

Using stable isotopes to track viruses in soils

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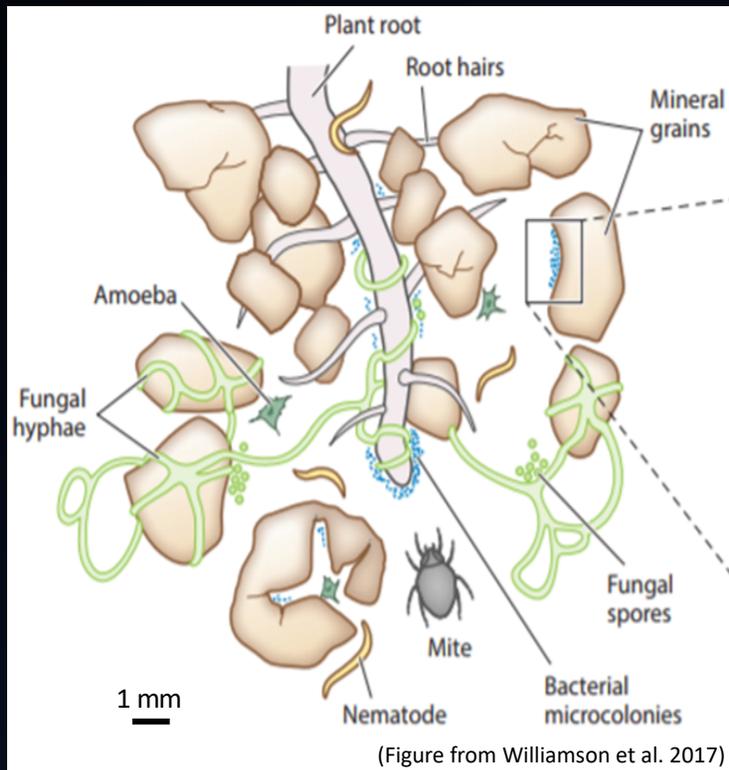
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344. Lawrence Livermore National Security, LLC



 Lawrence Livermore
National Laboratory

Soils are complex ecosystems

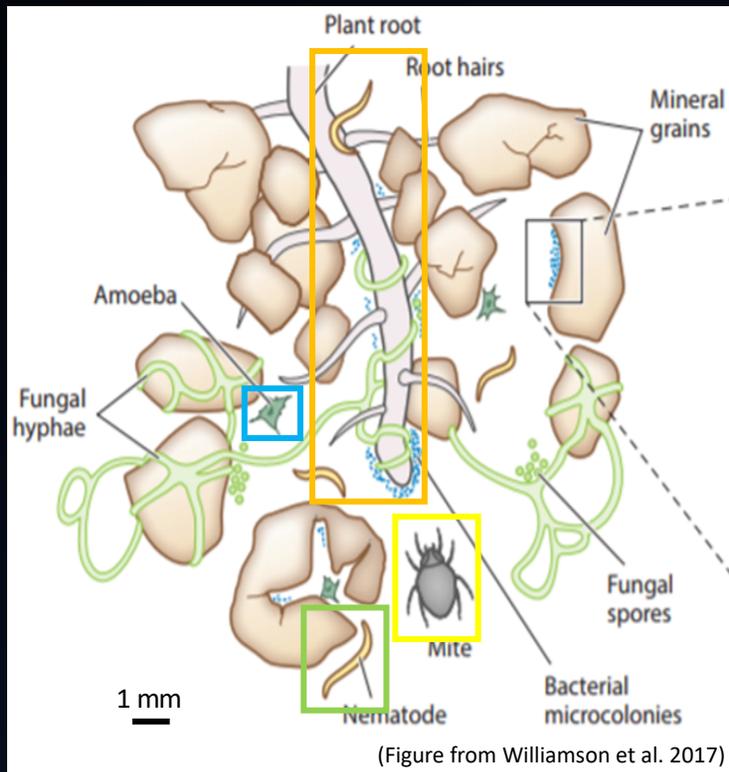
- Most insights come from isolates & metagenomics



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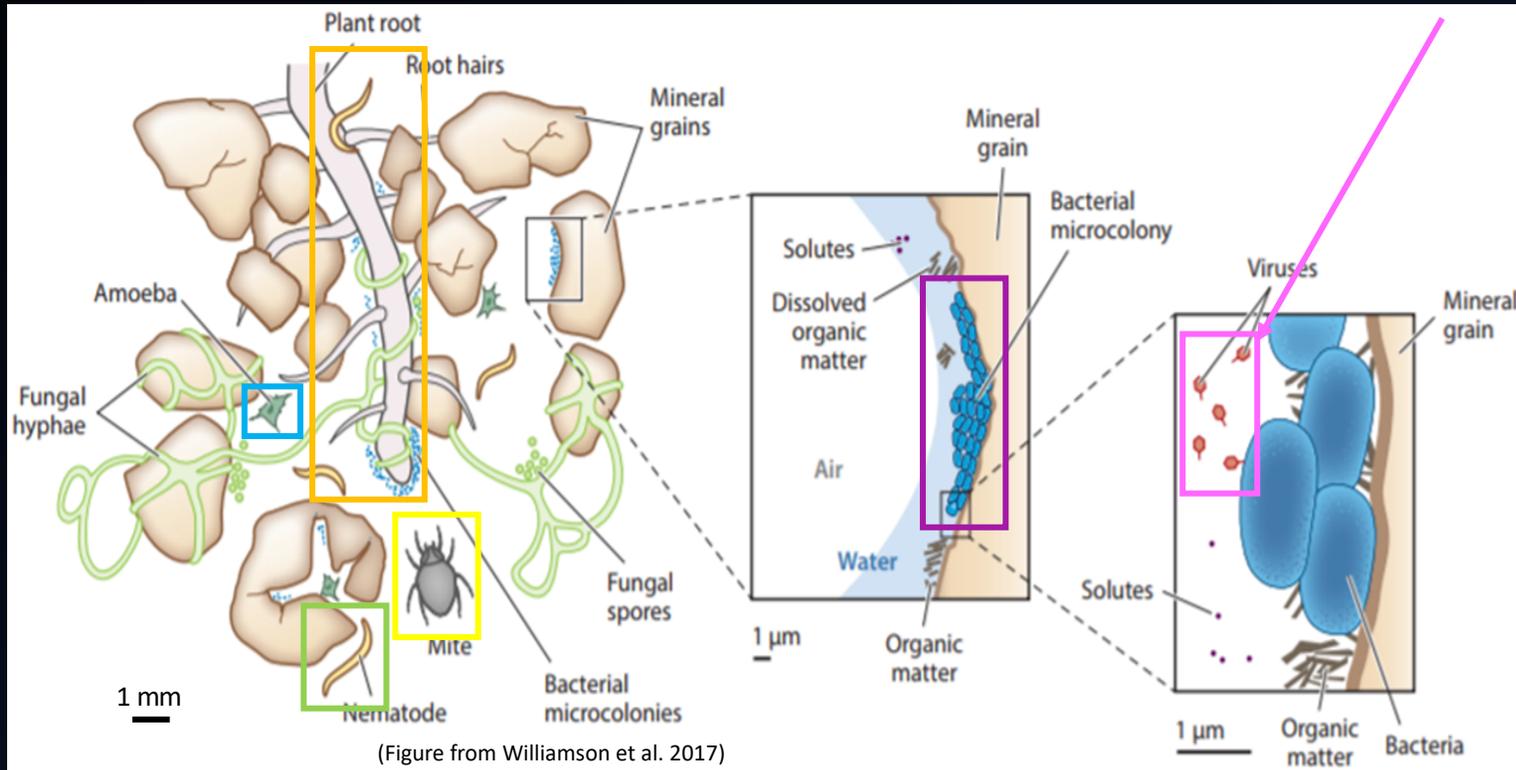
Plants Macrofauna Mesofauna Microfauna



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Plants Macrofauna Mesofauna Microfauna Microbes Viruses

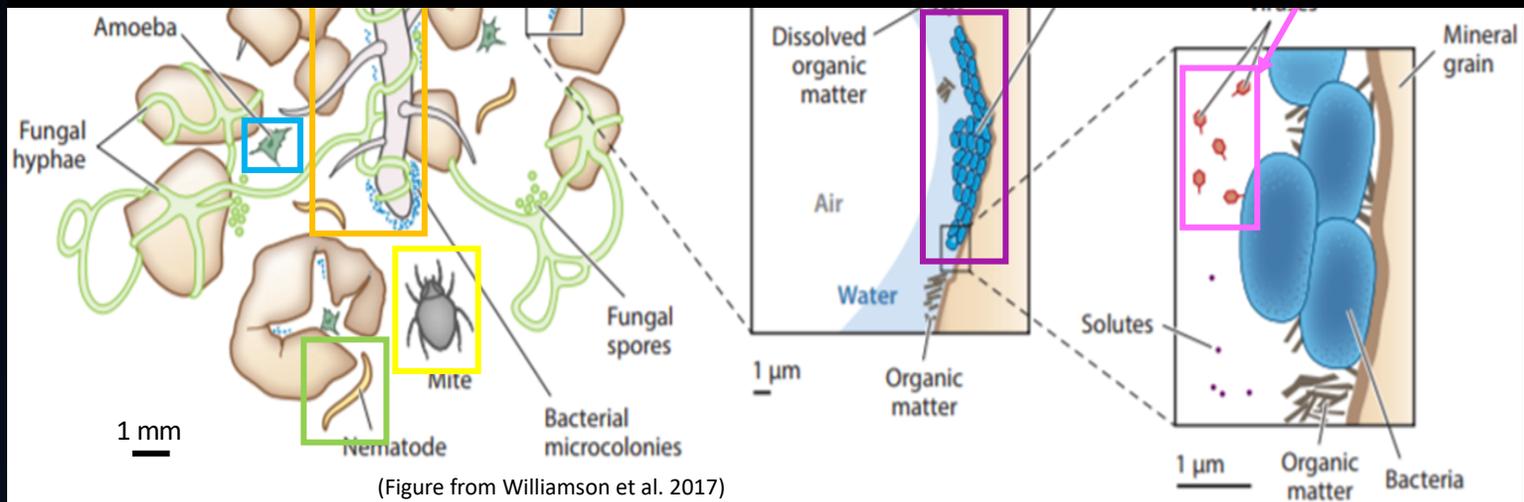


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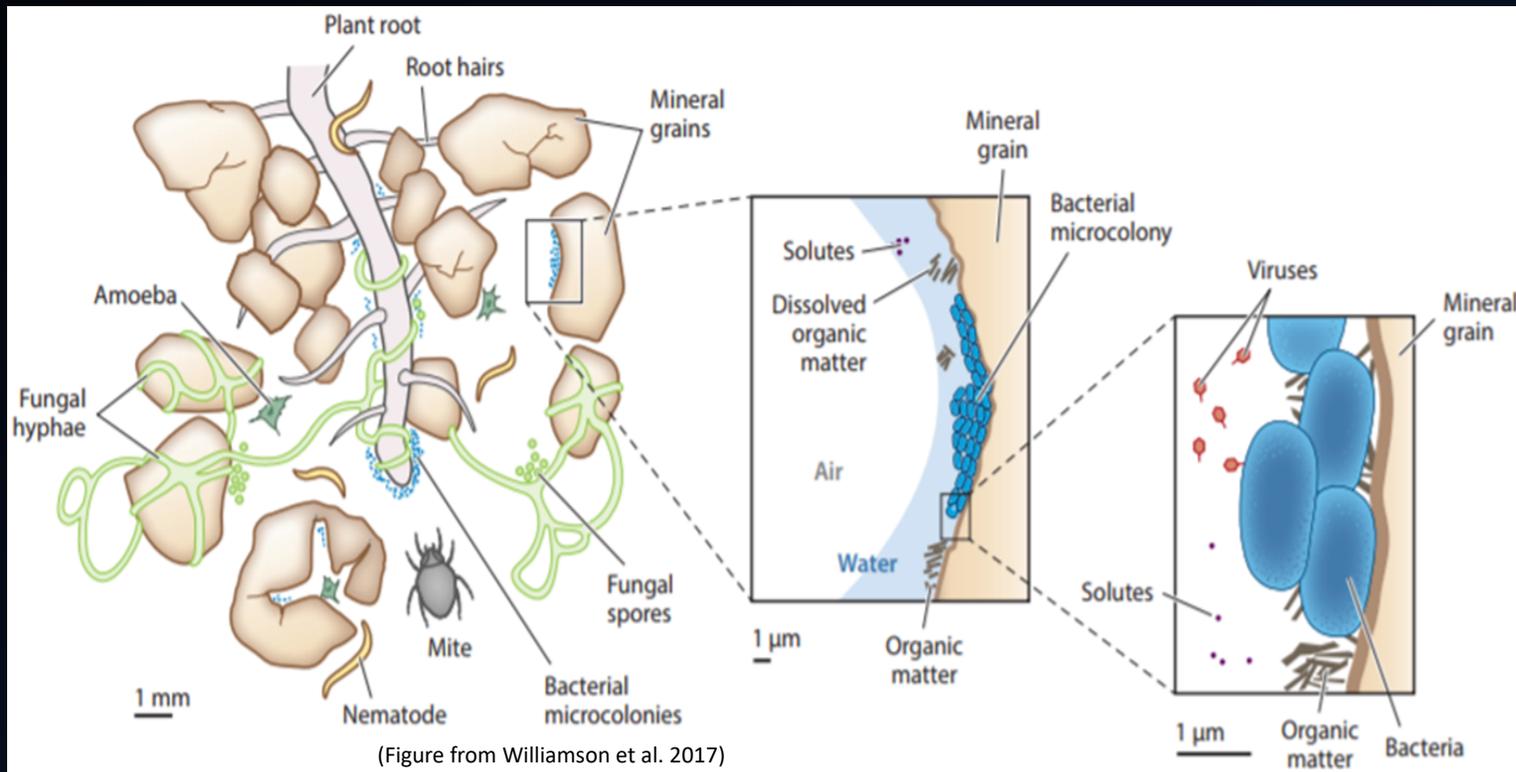
Plants Macrofauna Mesofauna Microfauna Microbes Viruses

Soil viruses are hard to isolate & metagenomes are swamped by larger genomes =
low resolution on viruses



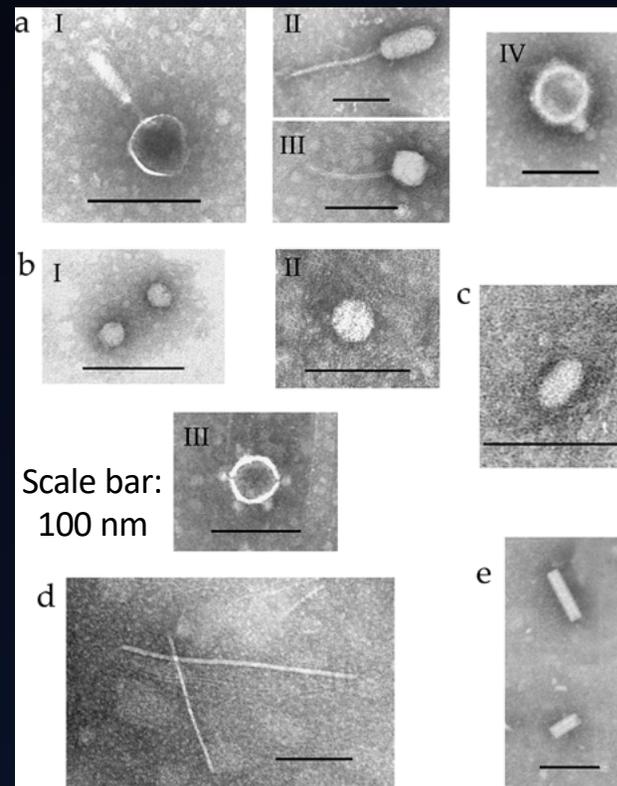
Limited Knowledge of Soil Viruses

🦠 10^7 – 10^9 viruses/g soil = ~ 1 – $1,000$ viruses: microbe



Limited Knowledge of Soil Viruses

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Limited Knowledge of Soil Viruses

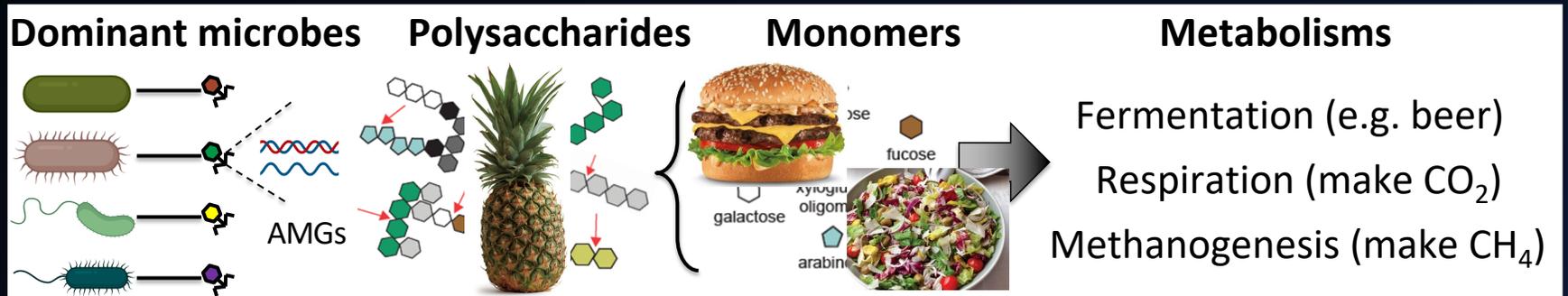
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 - 🔍 Recovery of thousands of viral populations (vOTUs; >10kb) (Emerson et al. 2018; Trubl et al. 2018; Trubl et al. 2019)

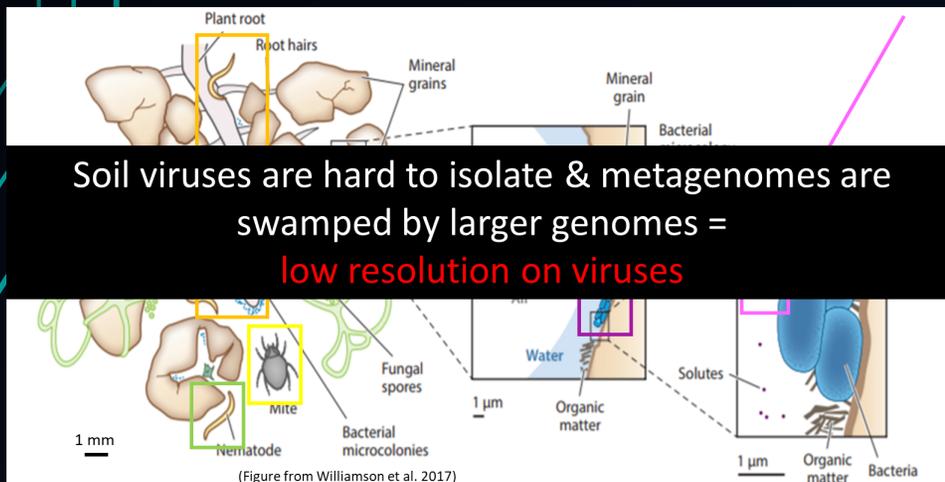
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 - Direct impacts on microbial biogeochemistry via lysis of dominant microbial lineages or expression of auxiliary metabolic genes (Emerson et al. 2018; Trubl et al. 2018)



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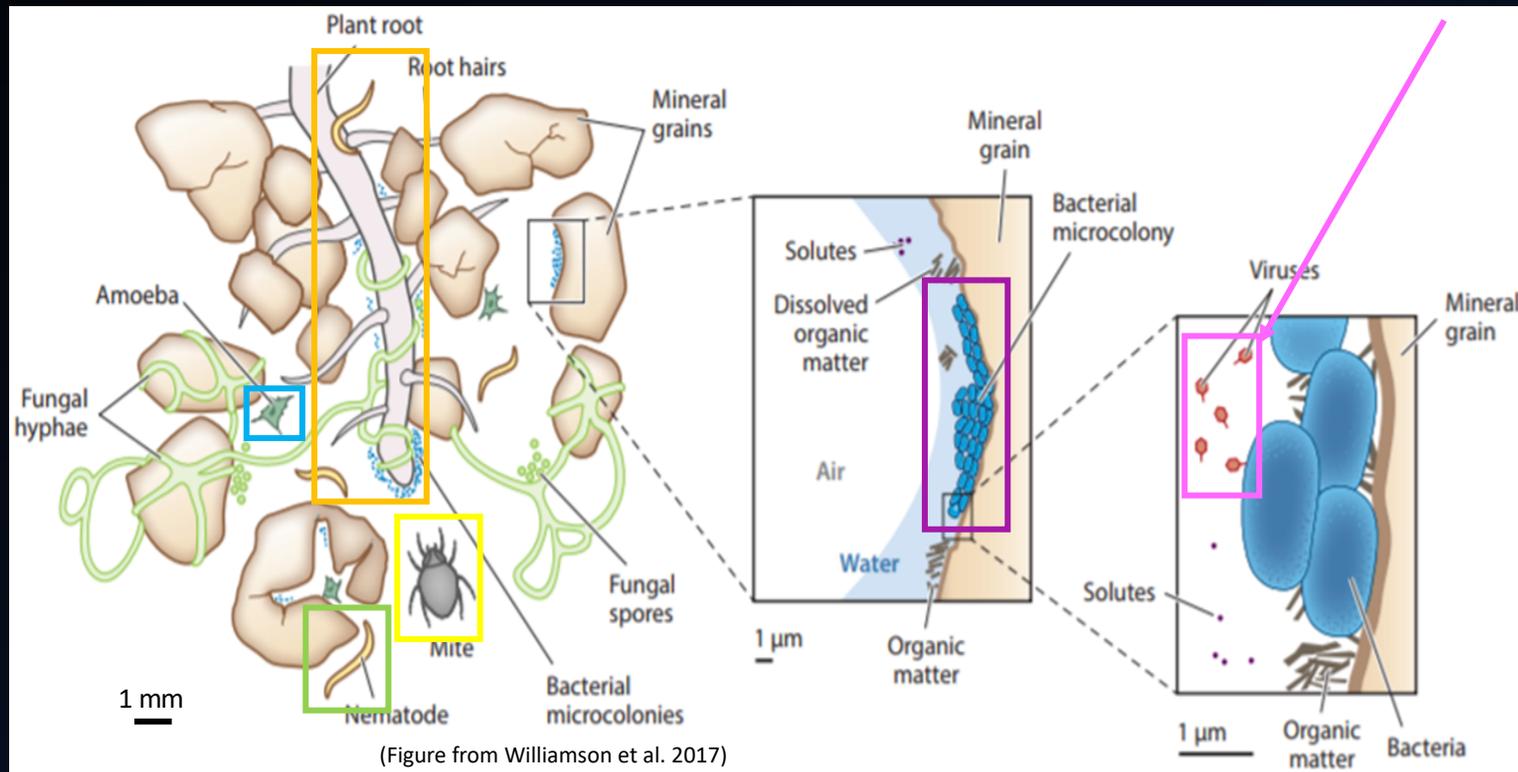


Recent advancements made possible by deep sequencing of metagenomes or laborious viromes

Soils are complex ecosystems

Most insights come from isolates & metagenomics

Plants Macrofauna Mesofauna Microfauna Microbes Viruses



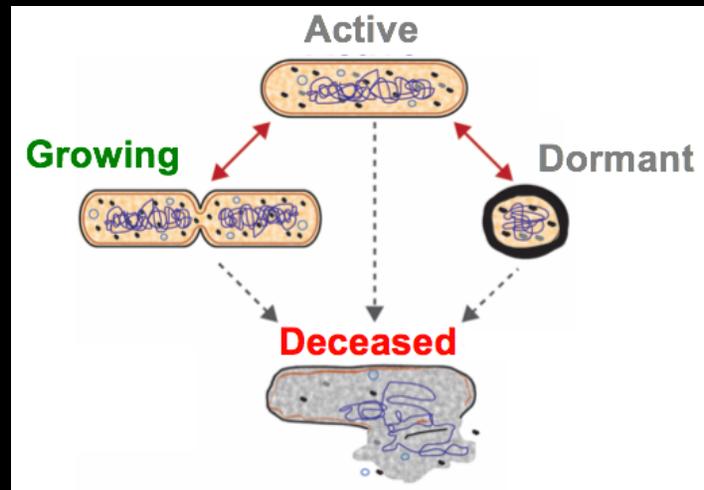
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Plants Macrofauna Mesofauna Microfauna Microbes Viruses

Plant root

Microbes exist in different metabolic states and majority of microbes are inactive in soil at a single time-point



(Figure from Williamson et al. 2017)

matter bacteria

Soils are complex ecosystems

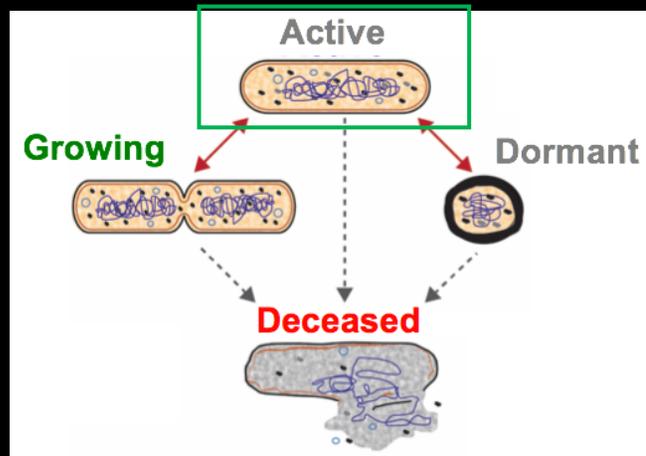
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Plants Macrofauna Mesofauna Microfauna Microbes Viruses

Plant root

Goals:

1. Increase resolution on viruses
2. Target active microbes and their viruses



How to track viruses?



Credit: Animal Humane Society



Credit: Derek Ramsey



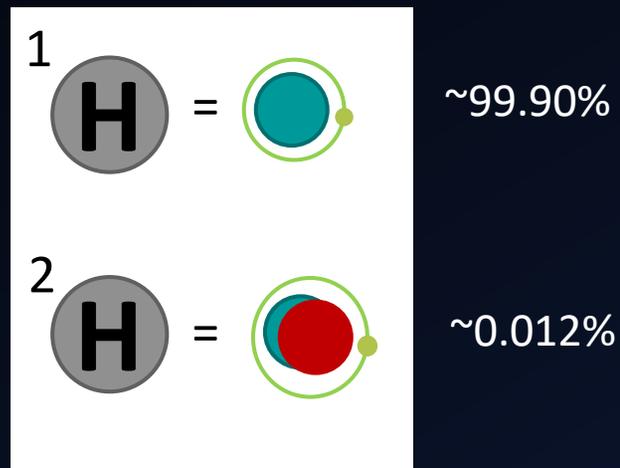
Credit: Carl Meyer

Viruses are too small...

Stable isotopes

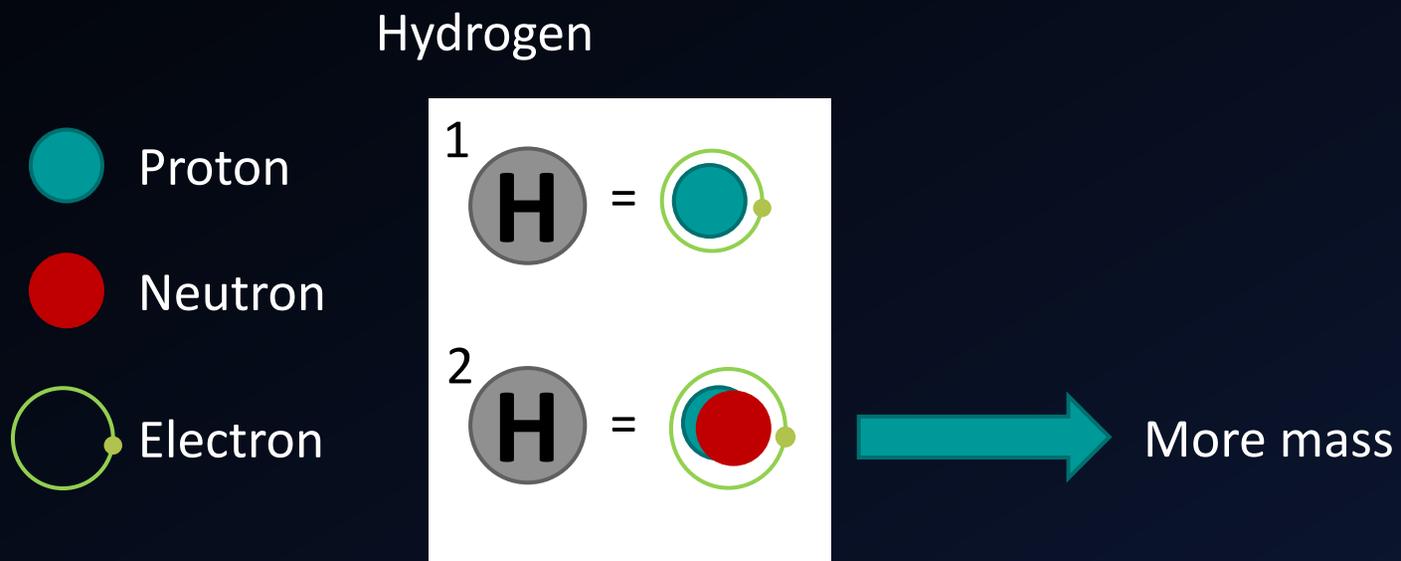
- Atoms that contain the same number of **protons** but differ in the number of **neutrons**

Hydrogen



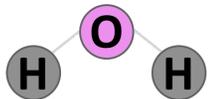
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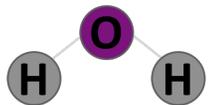
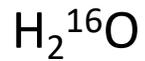


Stable isotopes

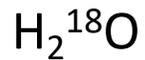
Can be tracers of biogeochemical processes



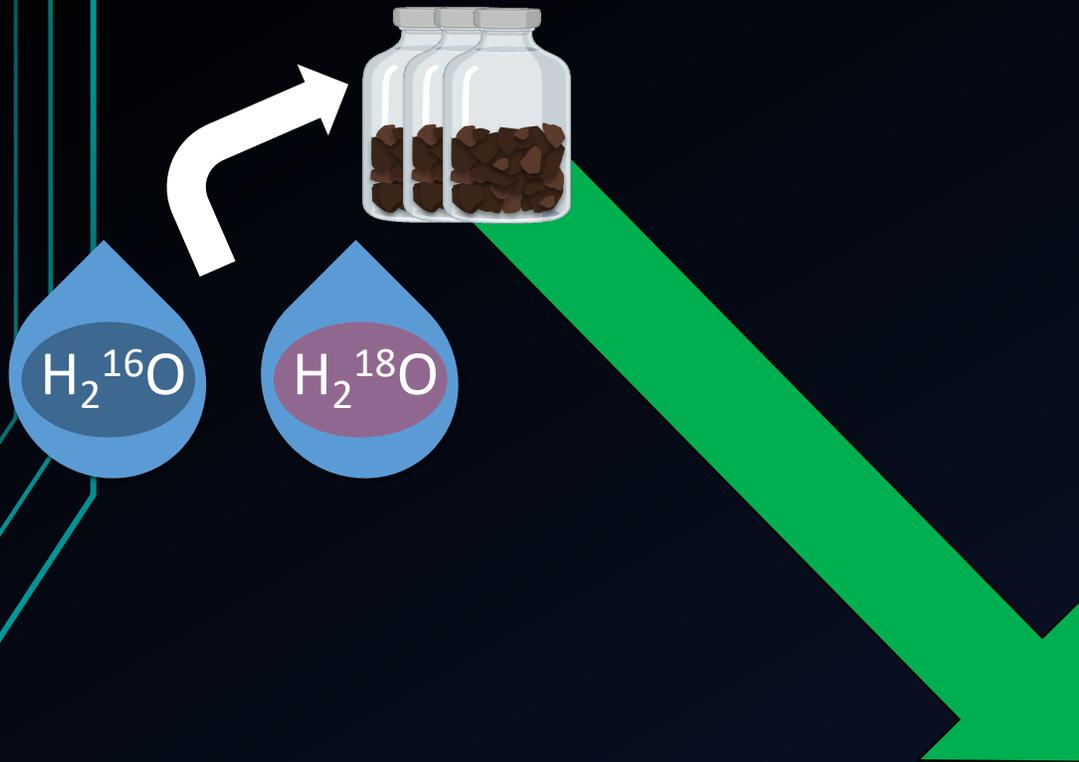
2 Hydrogens and 1 oxygen (8 protons/8 neutrons)



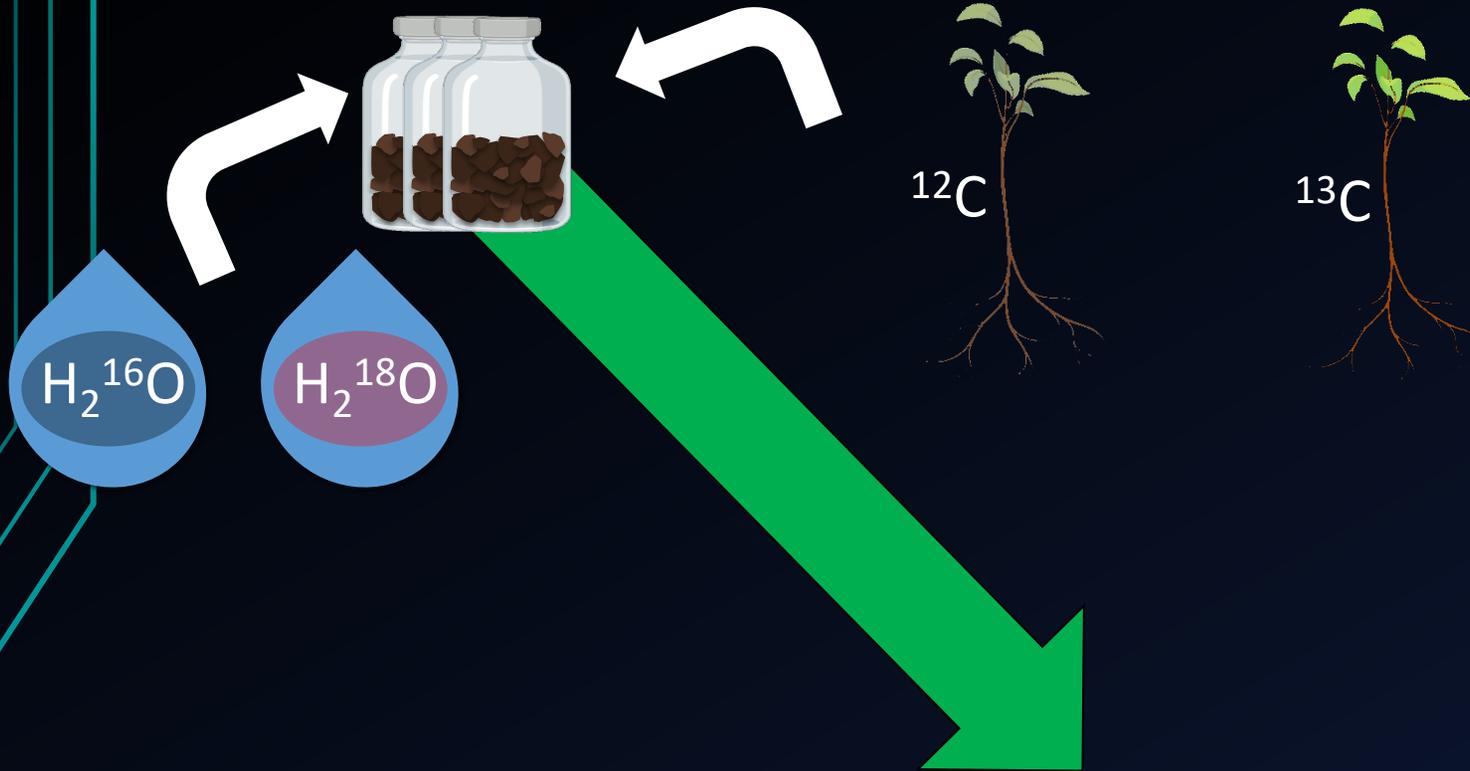
2 Hydrogens and 1 oxygen (8 protons/10 neutrons)



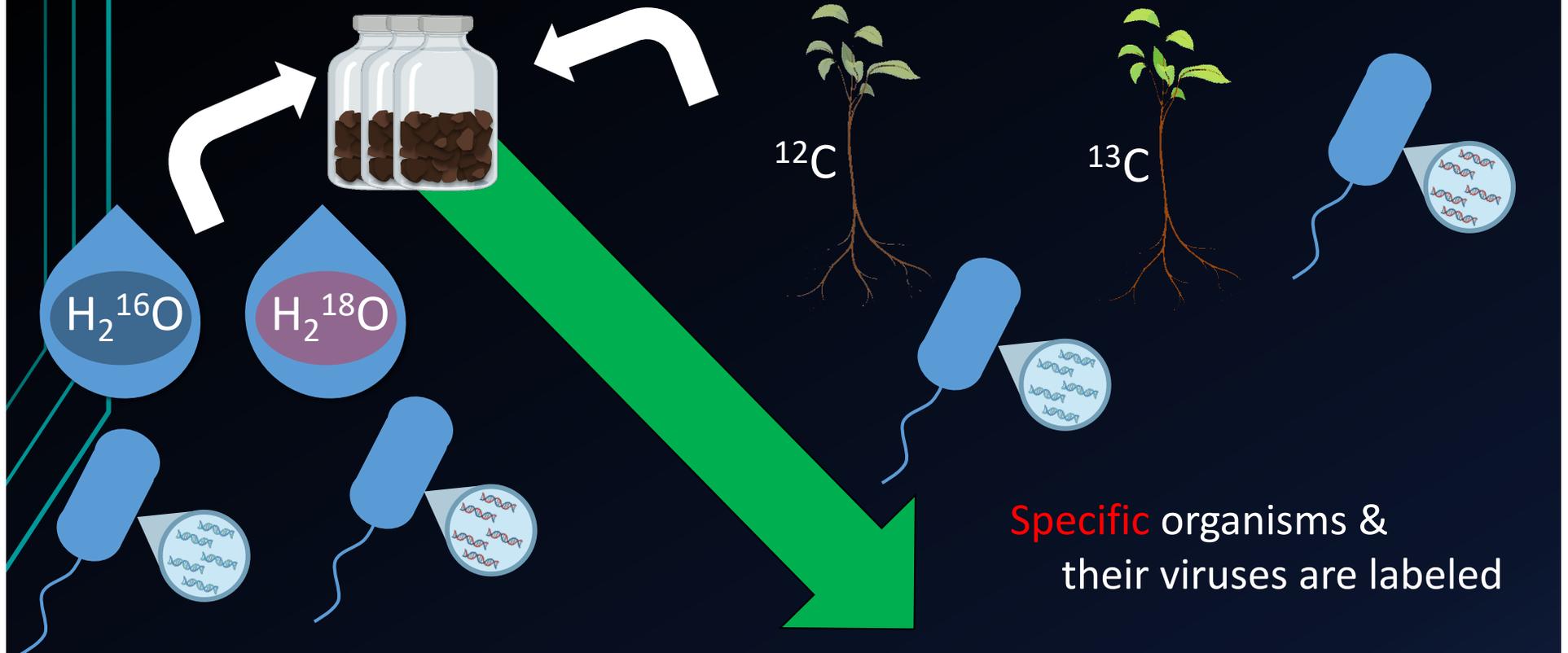
Characterizing viruses via targeted SIP-metagenomics



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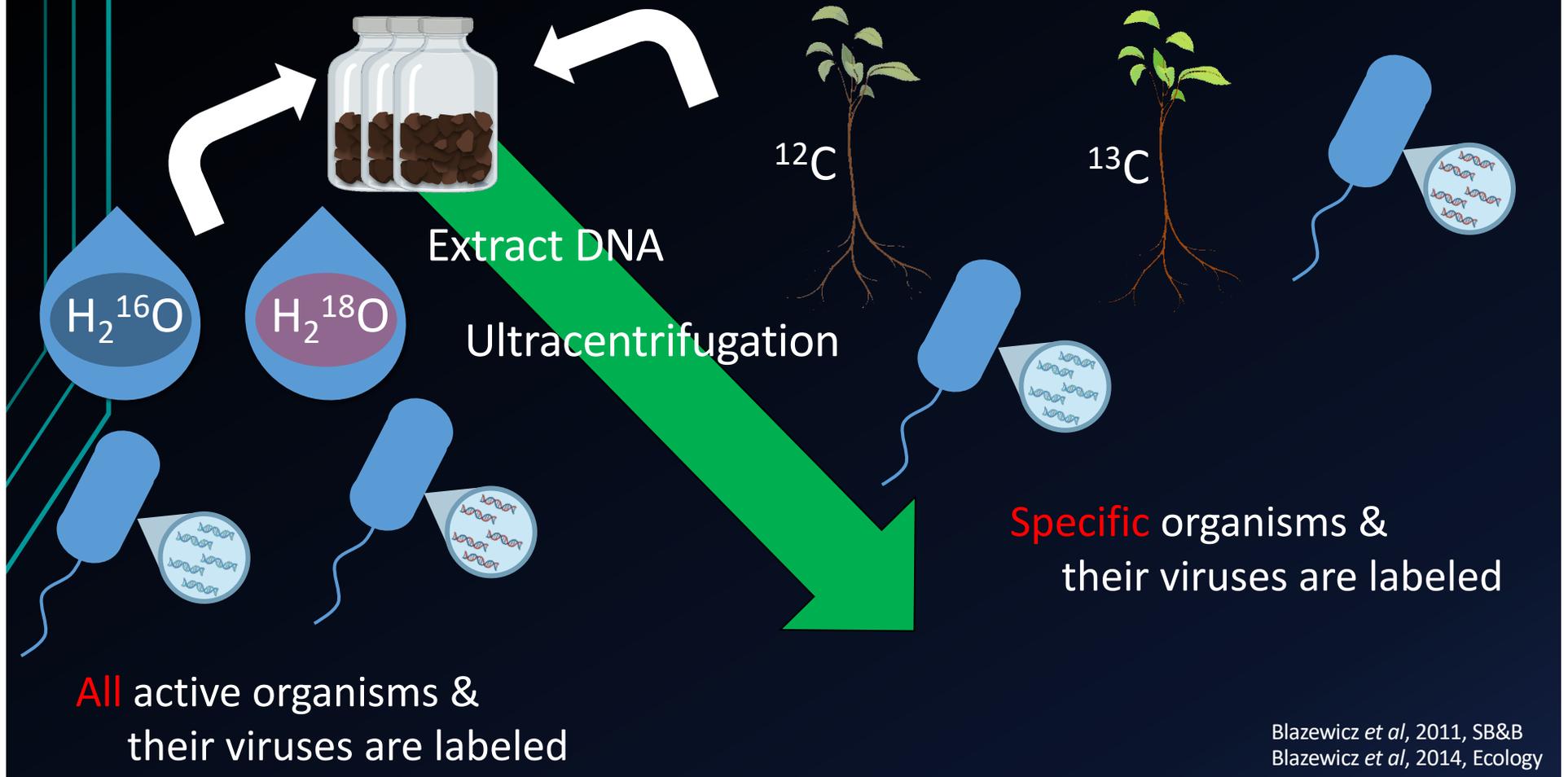
Characterizing viruses via targeted SIP-metagenomics



All active organisms &
their viruses are labeled

Specific organisms &
their viruses are labeled

Characterizing viruses via targeted SIP-metagenomics

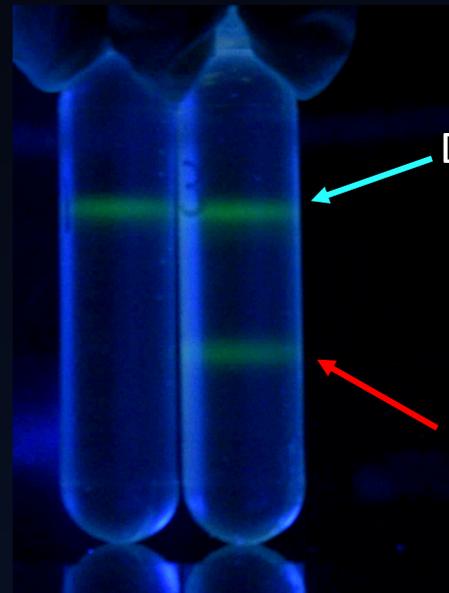


Characterizing viruses via targeted SIP-metagenomics



Extract DNA

Ultracentrifugation



Dormant
or deceased

New growth

Characterizing viruses via targeted SIP-metagenomics



Extract DNA

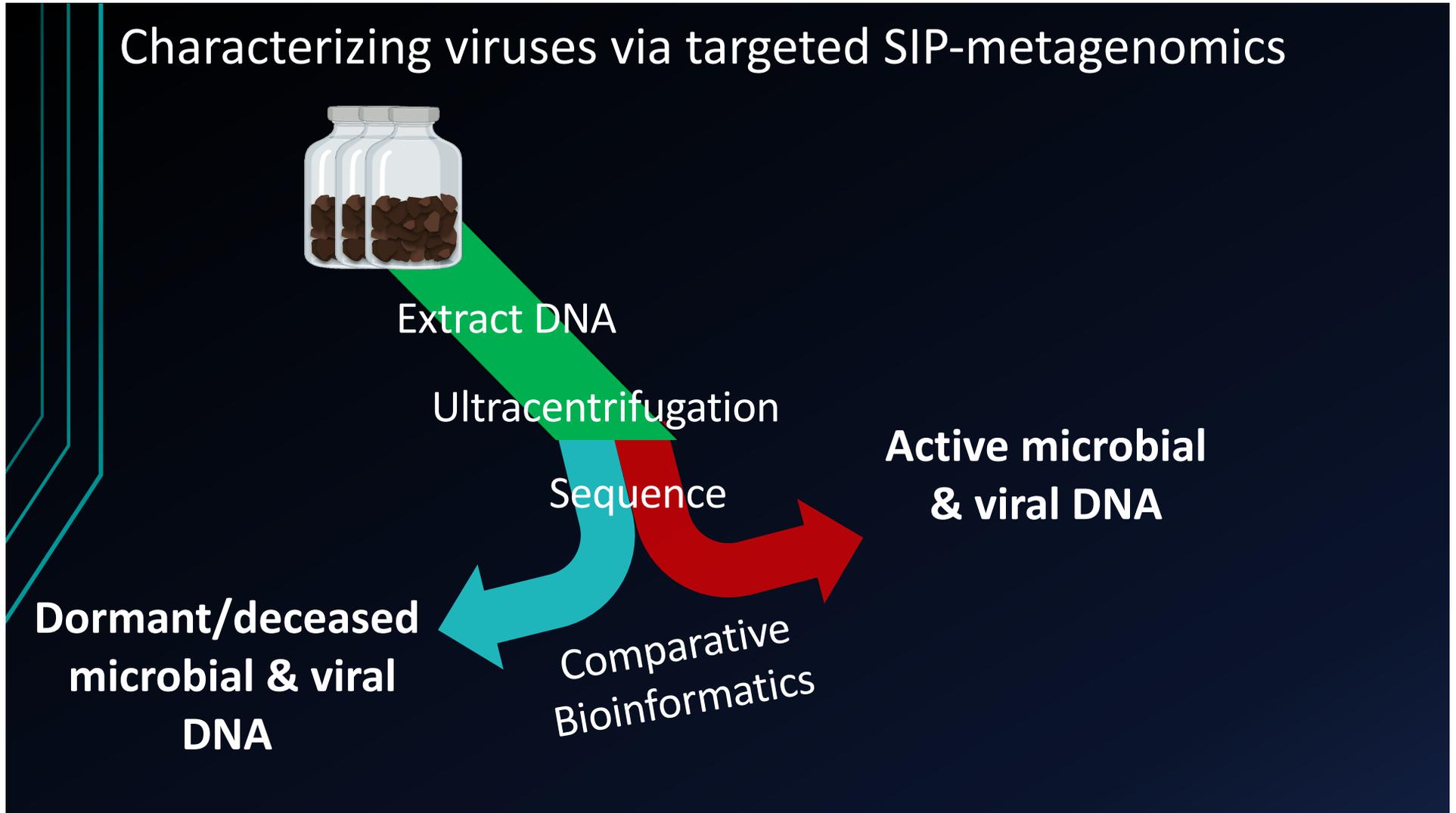
Ultracentrifugation

Sequence

**Active microbial
& viral DNA**

**Dormant/deceased
microbial & viral
DNA**

Comparative
Bioinformatics



Characterizing viruses via targeted SIP-metagenomics



Extract DNA

Ultracentrifugation

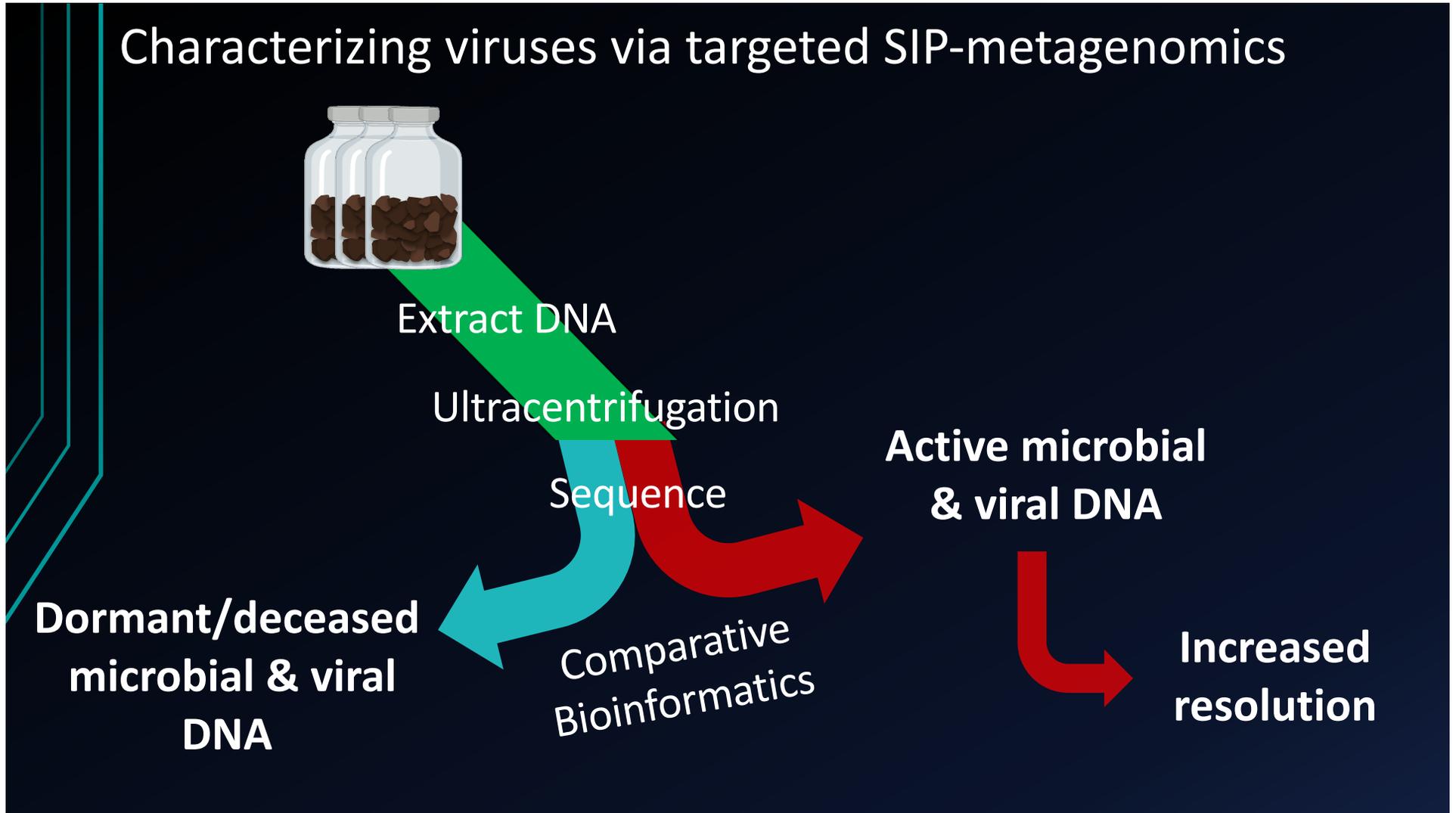
Sequence

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Bioinformatics

Increased
resolution



Characterizing viruses via targeted SIP-metagenomics



Extract DNA

Goals:

- ✓ 1. Increase resolution on viruses
- ✓ 2. Target active microbes and their viruses

Microbial
DNA

Dormant/deceased
microbial & viral
DNA

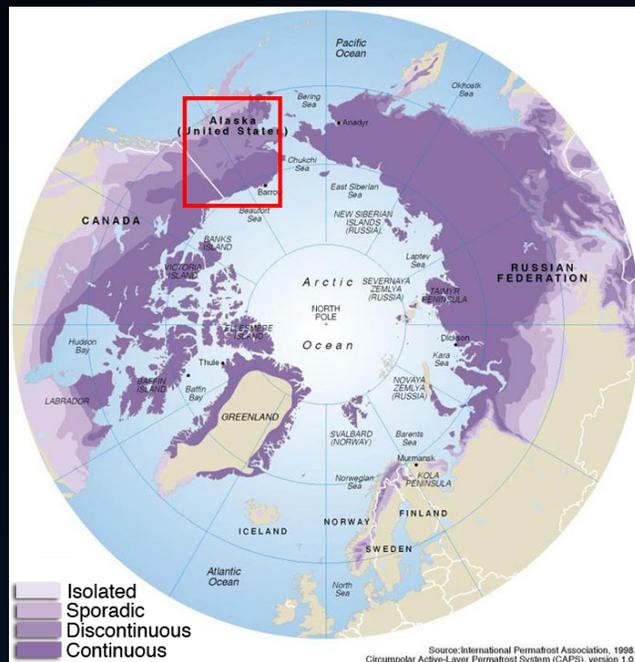
Comparative
Bioinformatics

Increased
resolution

Applying SIP to Metagenomics in different biomes

- 1) Characterize diversity of dsDNA viruses in soil from different biomes
- 2) Identify active viruses and their microbial hosts
 - Two Long-term ecological research (LTER) sites
 - Partially-thaw permafrost bog habitat
 - Highly-dynamic tropical rainforest

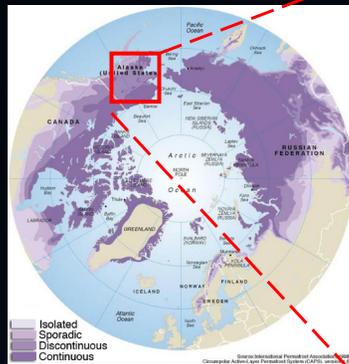
Bonanza Creek, Alaska



Source: <https://www.lter.uaf.edu/>

Bonanza Creek, Alaska

Avg. temperature
-3.1°C



Source: <https://www.lter.uaf.edu/>

Bonanza Creek, Alaska



Source: <https://www.lter.uaf.edu/>

Experimental overview



Anoxic at 4°C

10 cm

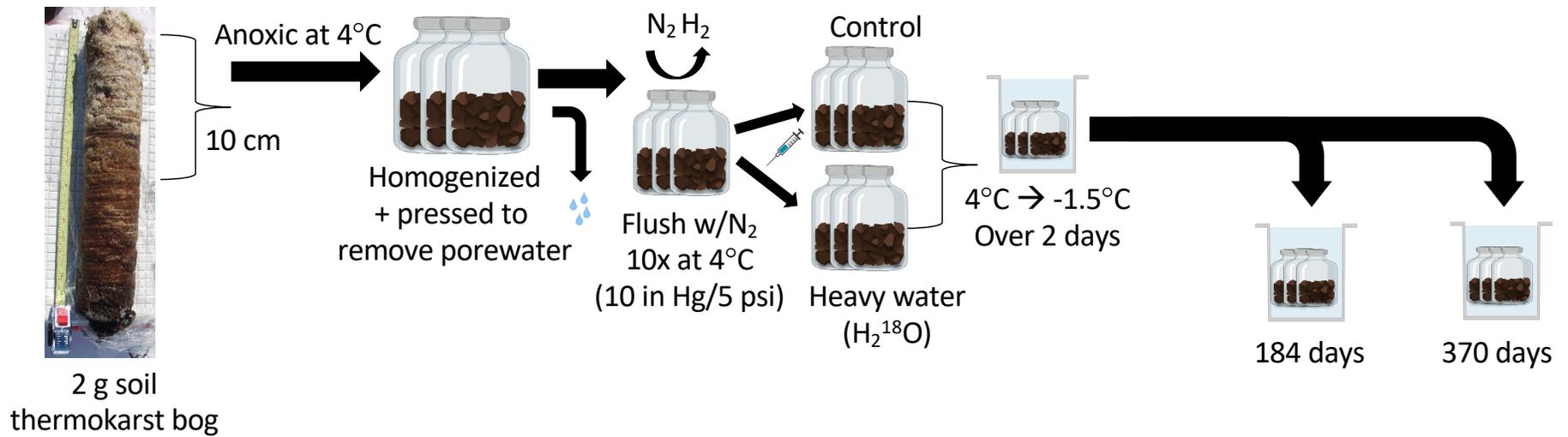


Homogenized
+ pressed to
remove porewater

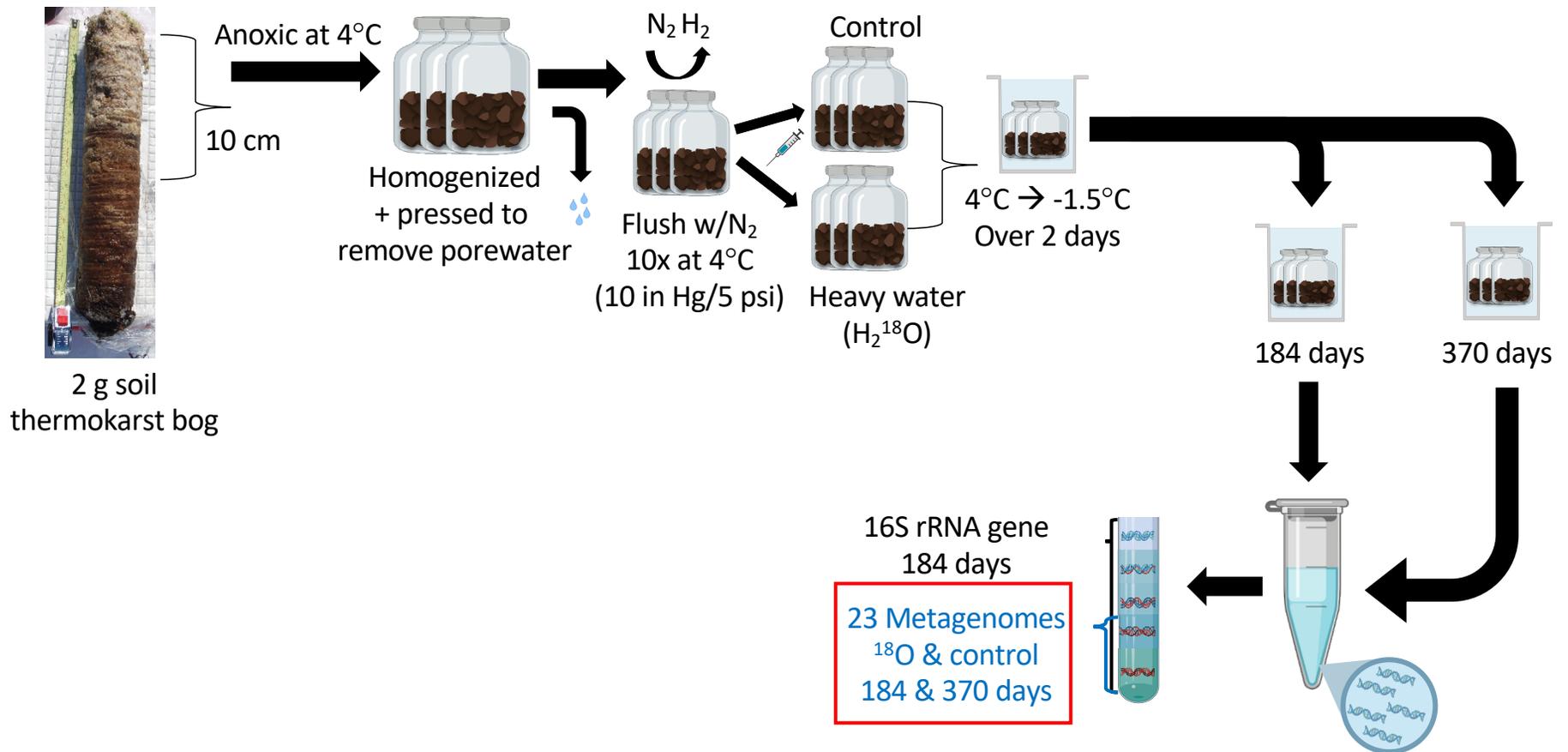


2 g soil
thermokarst bog

Experimental overview



Experimental overview



Bonanza Creek, Alaska

Overview

- 23 metagenomes

30 active metagenome-assembled genomes (MAGs)

Bonanza Creek, Alaska

Overview

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30 active metagenome-assembled genomes (MAGs)

Bacterial hosts spanned 3 phyla:

Proteobacteria

Bacteroidetes

Firmicutes

Bonanza Creek, Alaska

Overview

- 23 metagenomes

Bacteria active below freezing!

Bonanza Creek, Alaska

Overview

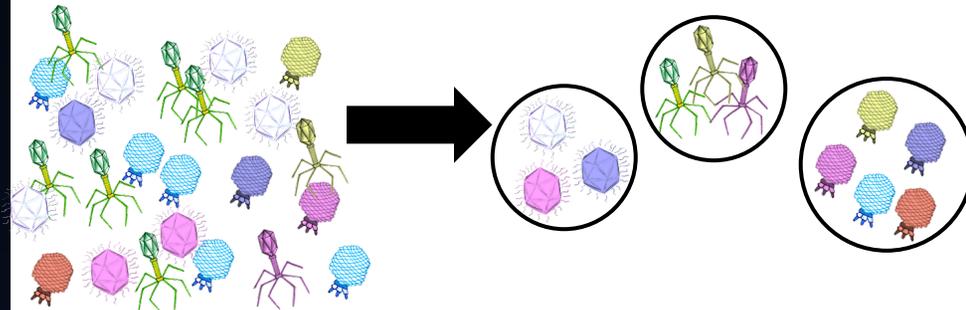
- 23 metagenomes
- ~4,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score ≥ 0.9 and P value < 0.05)

Bonanza Creek, Alaska

Overview

- 23 metagenomes
- ~4,000 viruses detected by VirSorter (categories 1 & 2) & DeepVirFinder (score ≥ 0.9 and P value < 0.05)
- 332 vOTUs ($\geq 10\text{kb}$)

Identifying viral populations ($>10\text{kb}$)

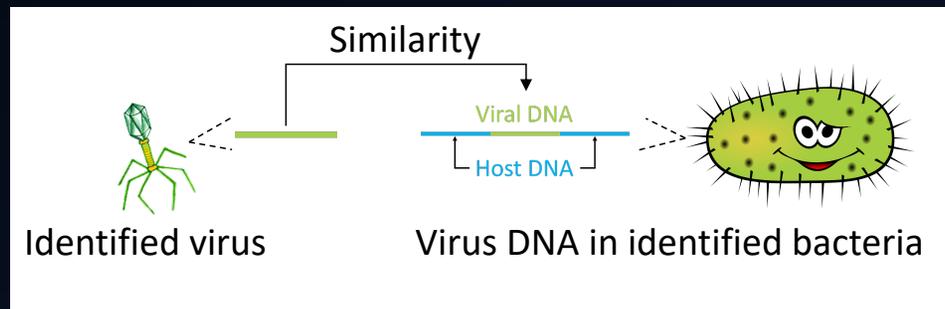


Identified viruses were clustered by 95% nucleotide identity over 85% of the shorter contig

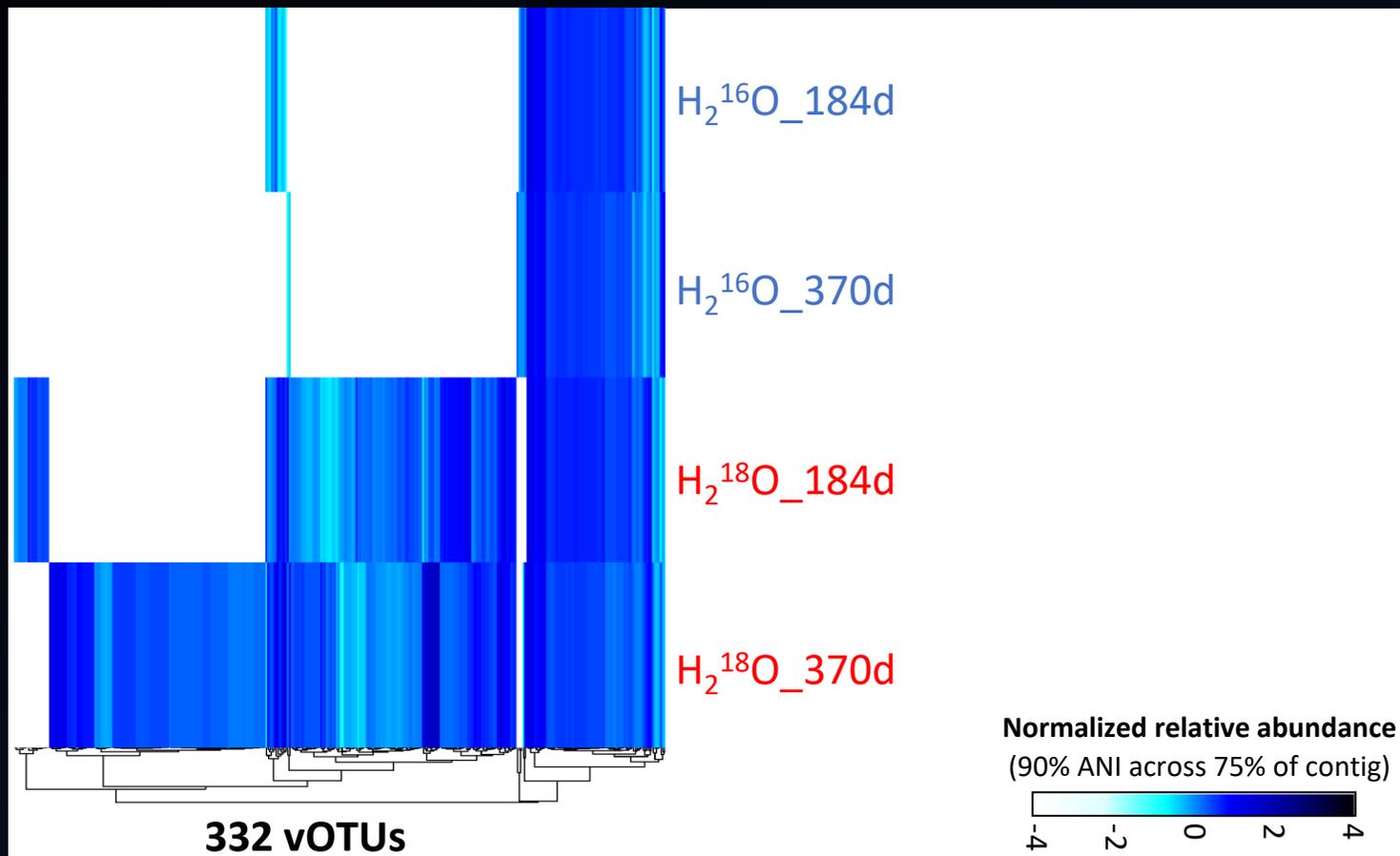
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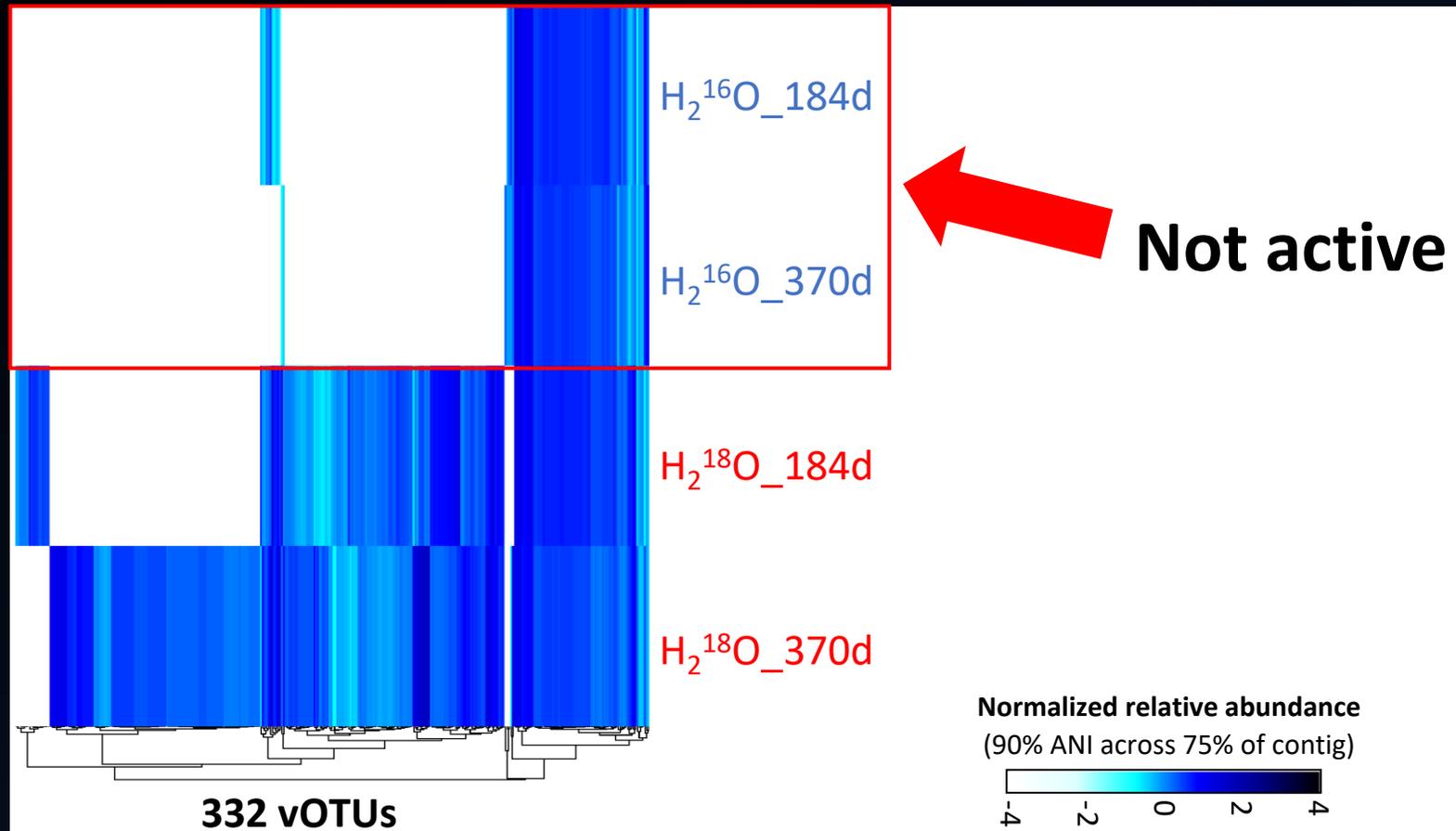
- 23 metagenomes
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- 332 vOTUs ($\geq 10\text{kb}$)
- 9 virus-host linkages via nucleotide identity (threshold 95% ANI & $\geq 1500\text{bp}$)...more to come



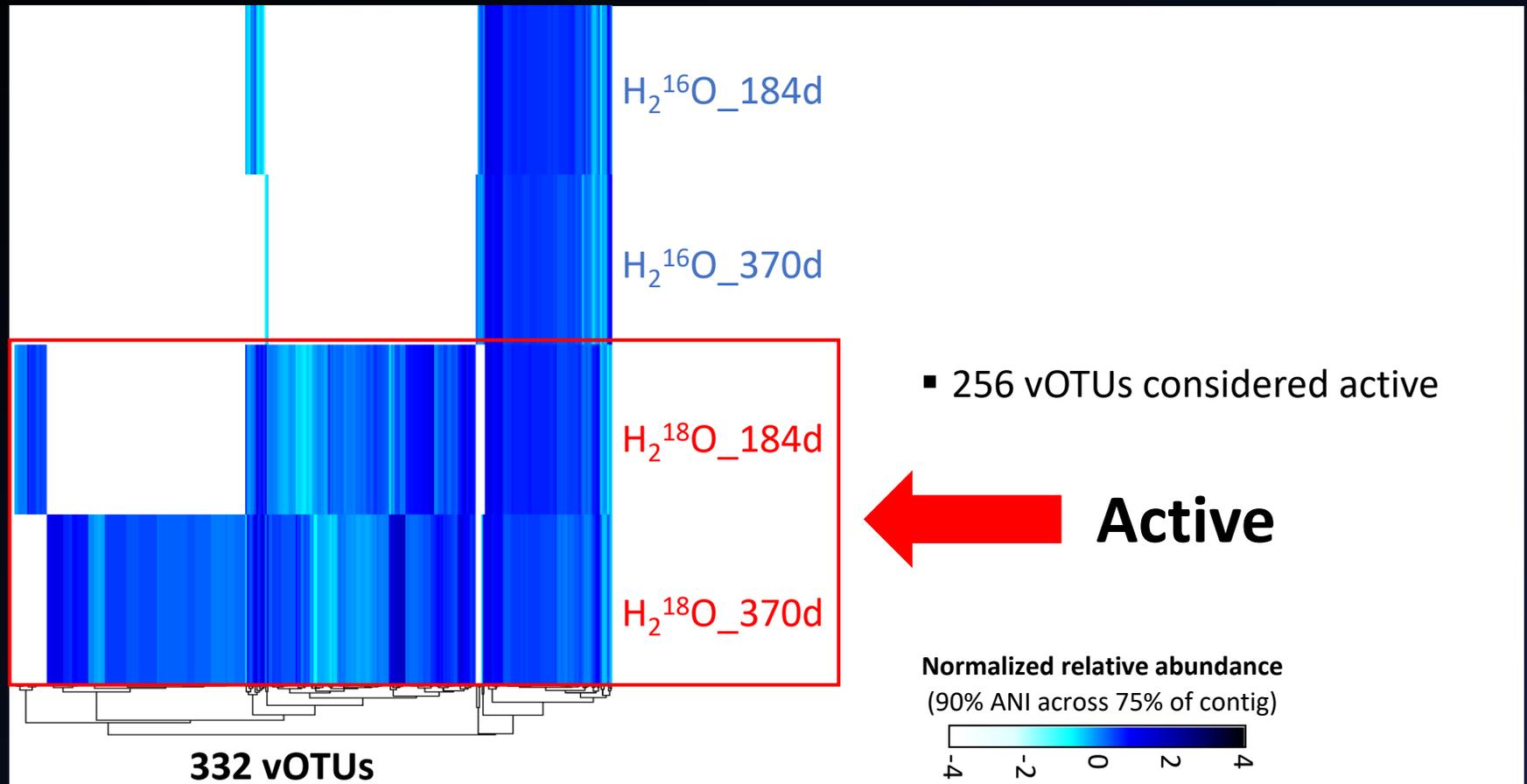
A lot of soil viruses detected!



Increased resolution by removing non-active viruses



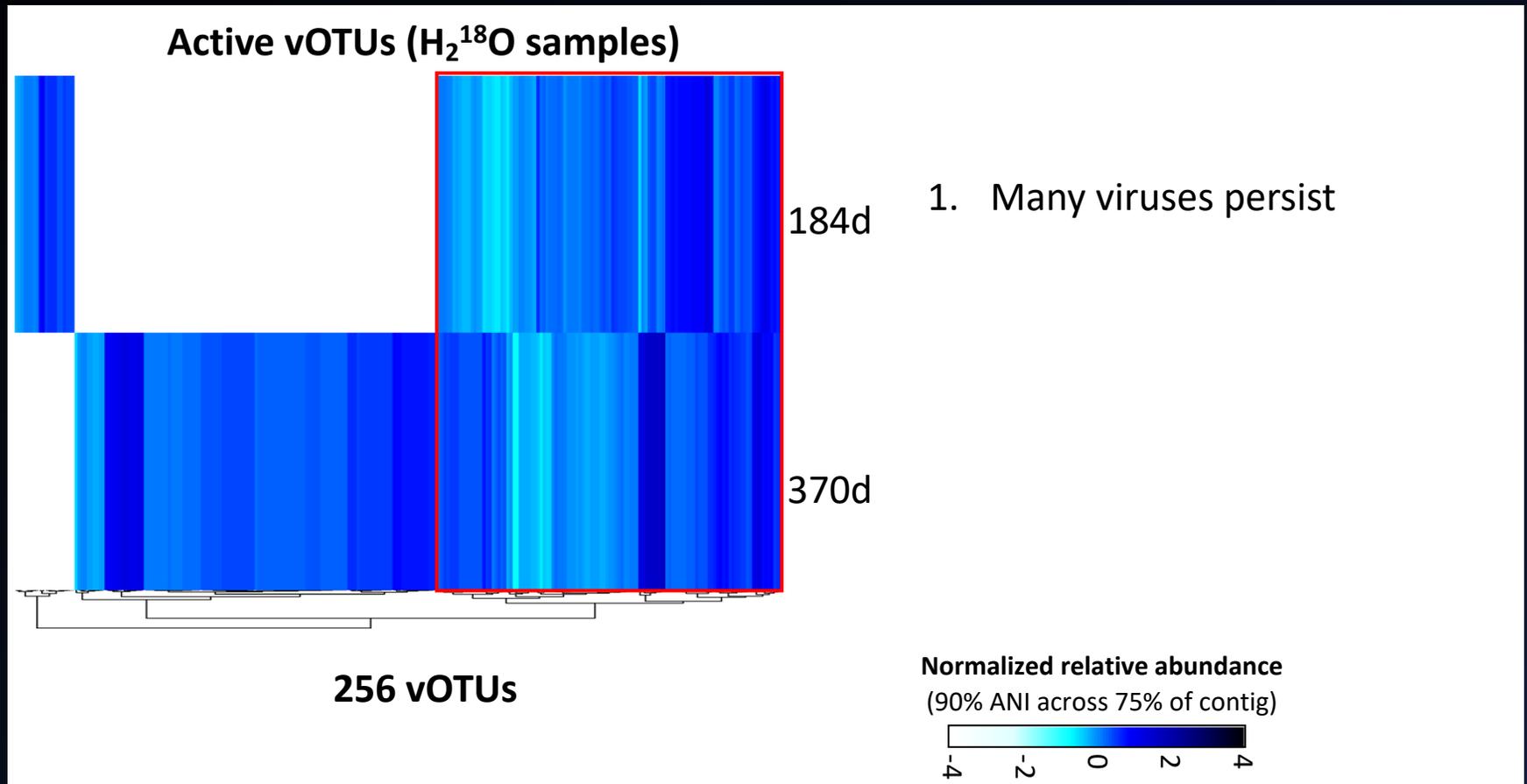
Many viruses are active



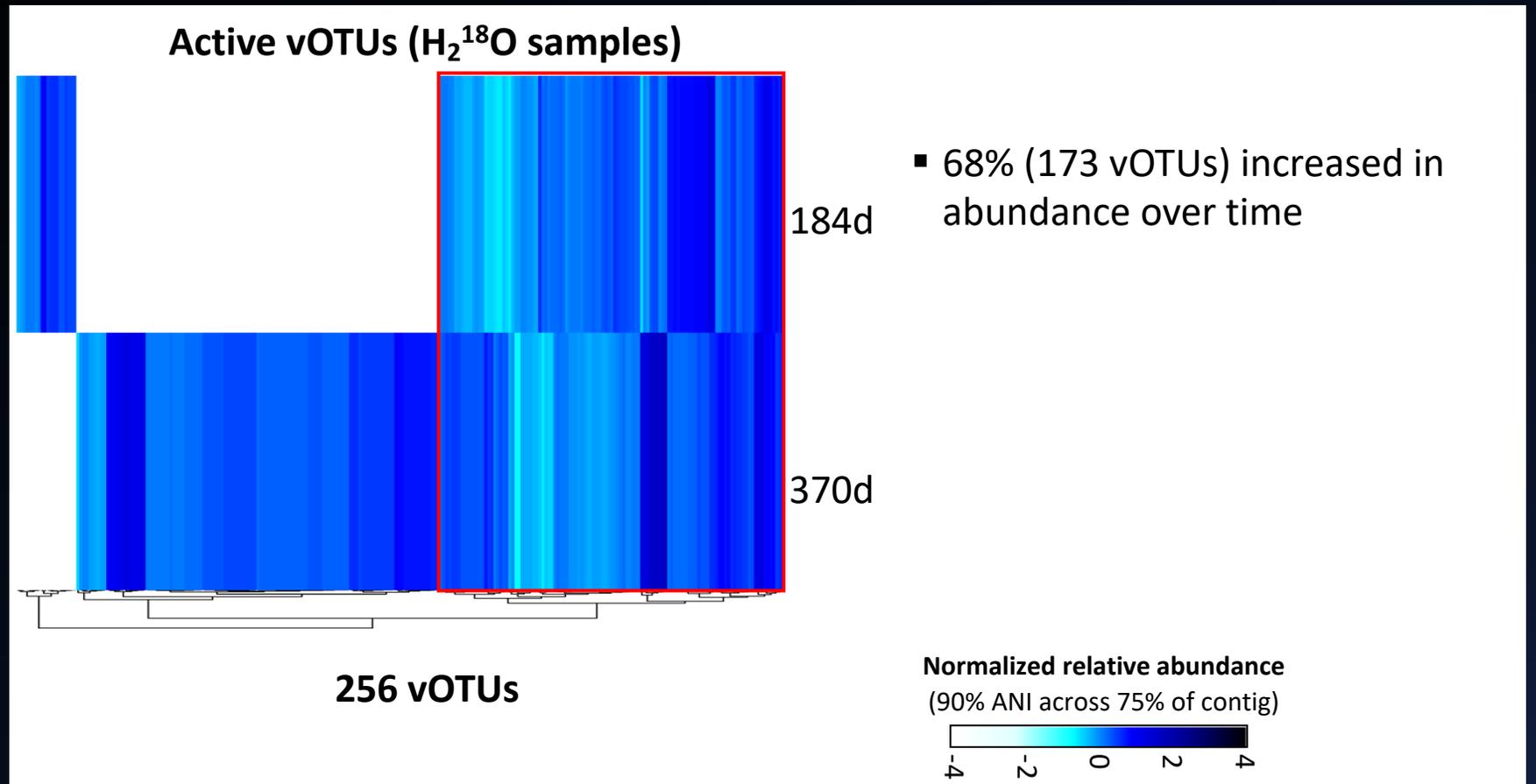
▪ 256 vOTUs considered active

Active

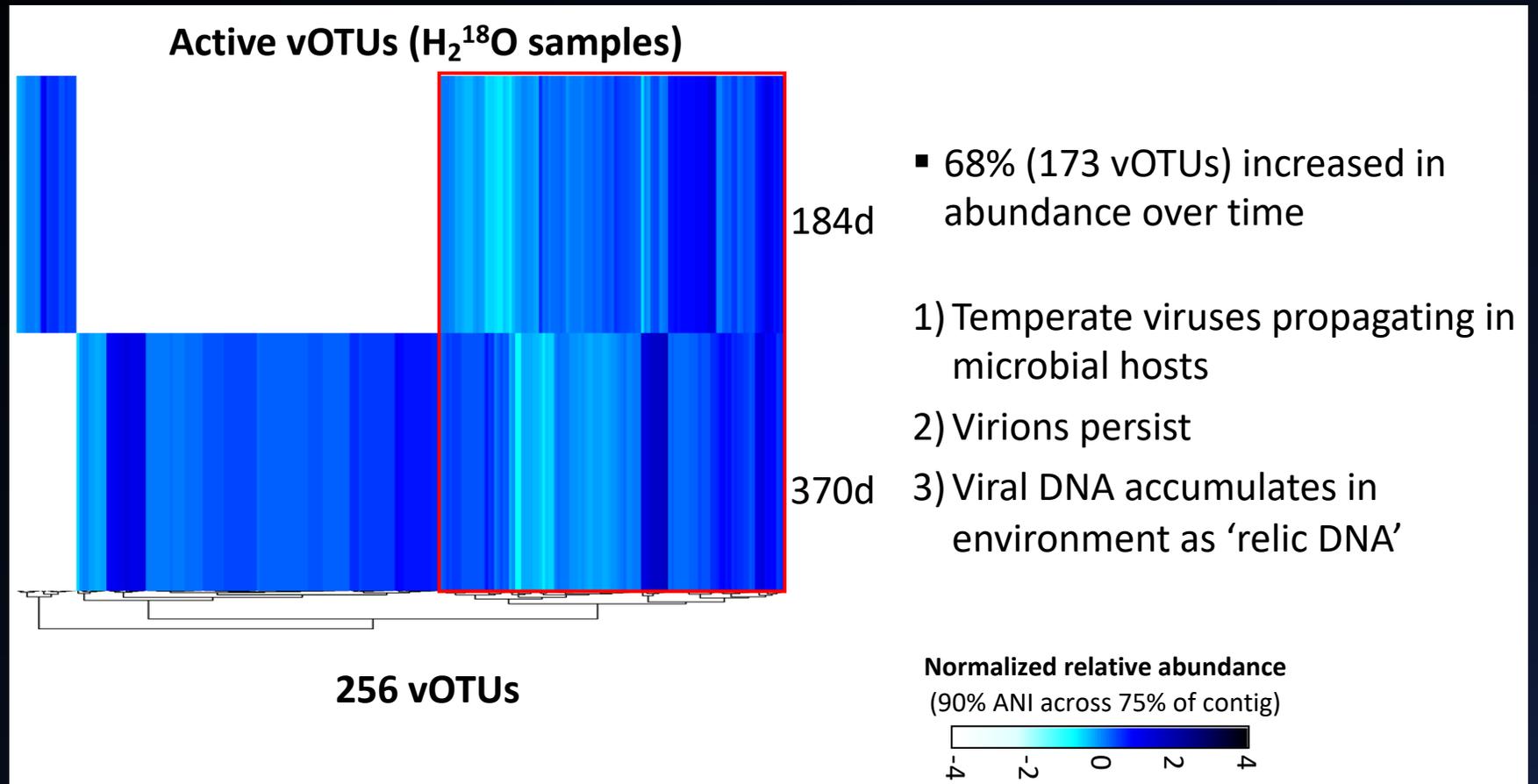
Viral community is dynamic



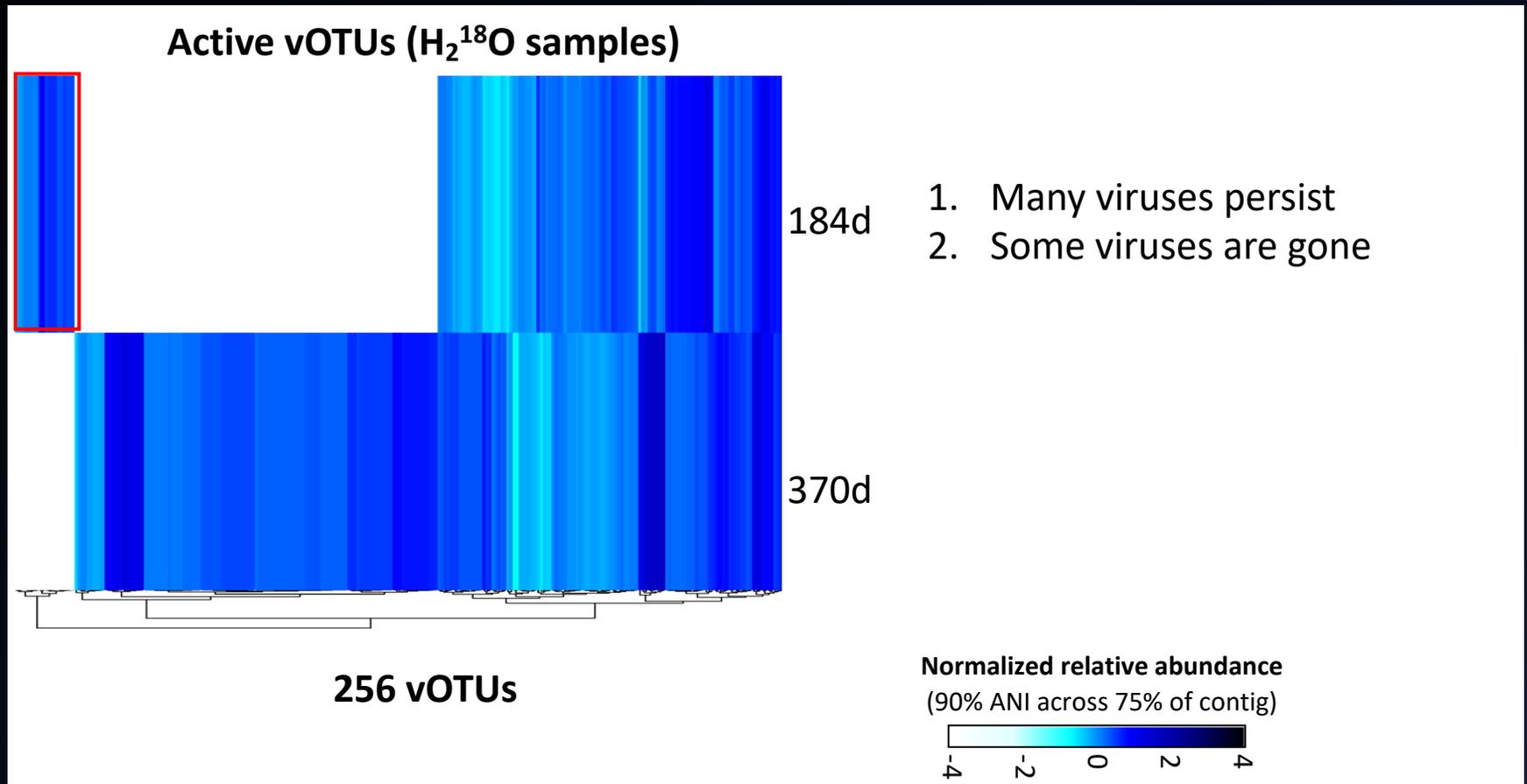
What does persistence mean, ecologically?



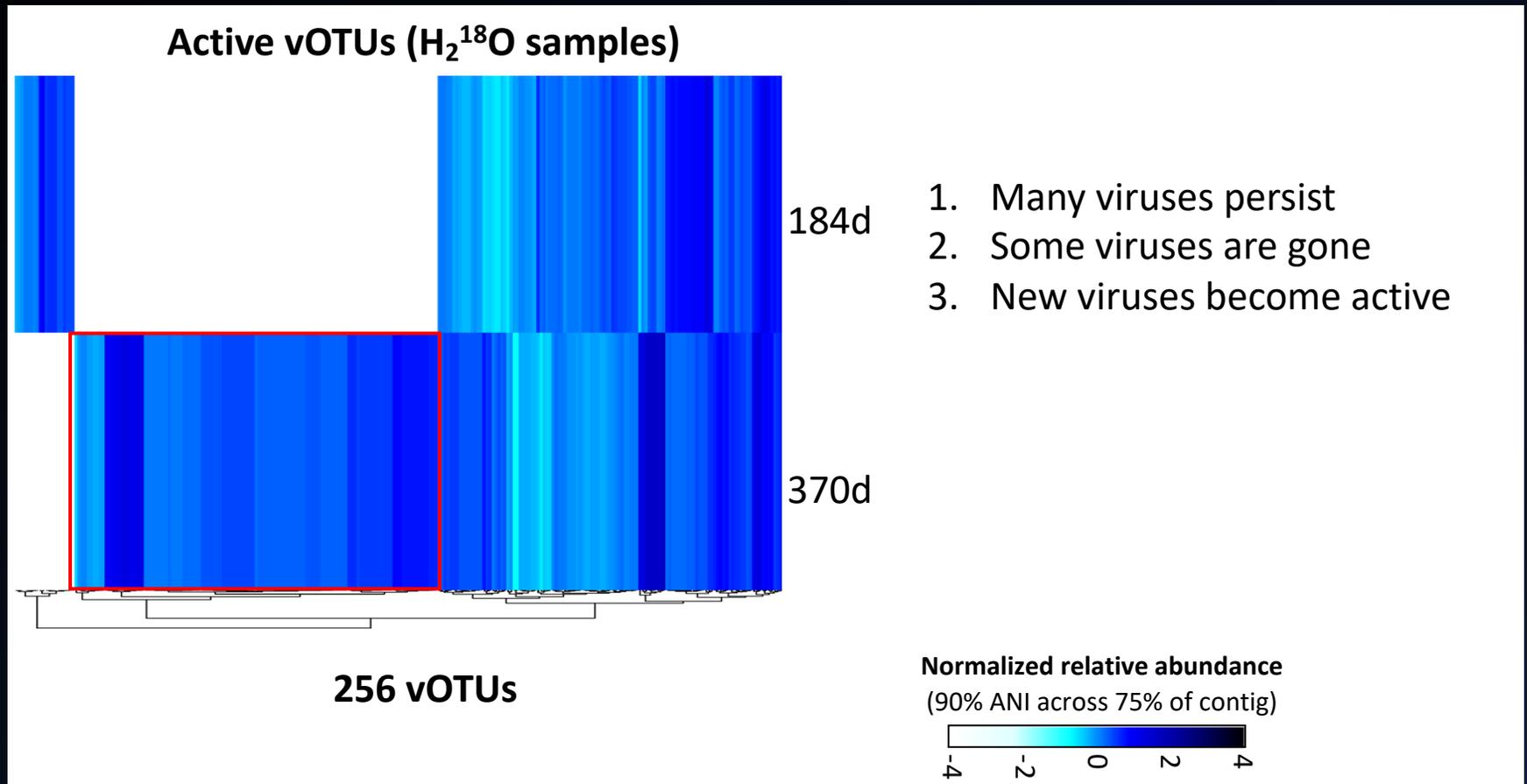
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Bonanza Creek, Alaska

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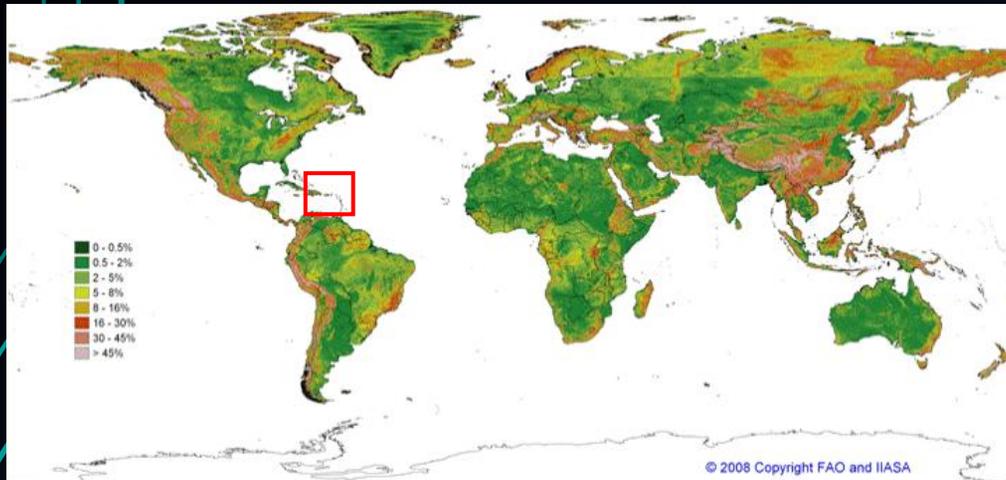
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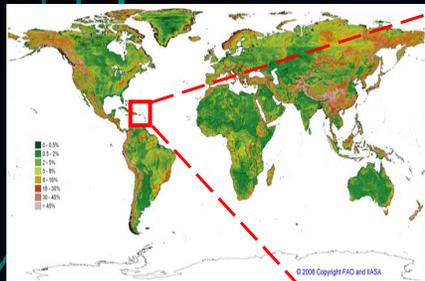
Luquillo Experimental Forest, Puerto Rico

- Soils naturally oscillate between oxic and anoxic conditions



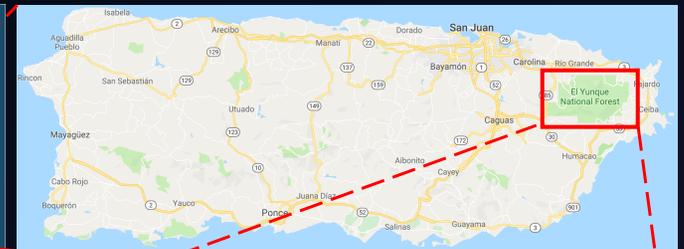
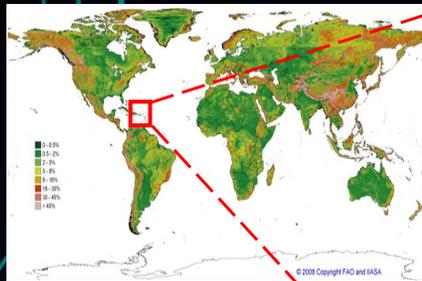
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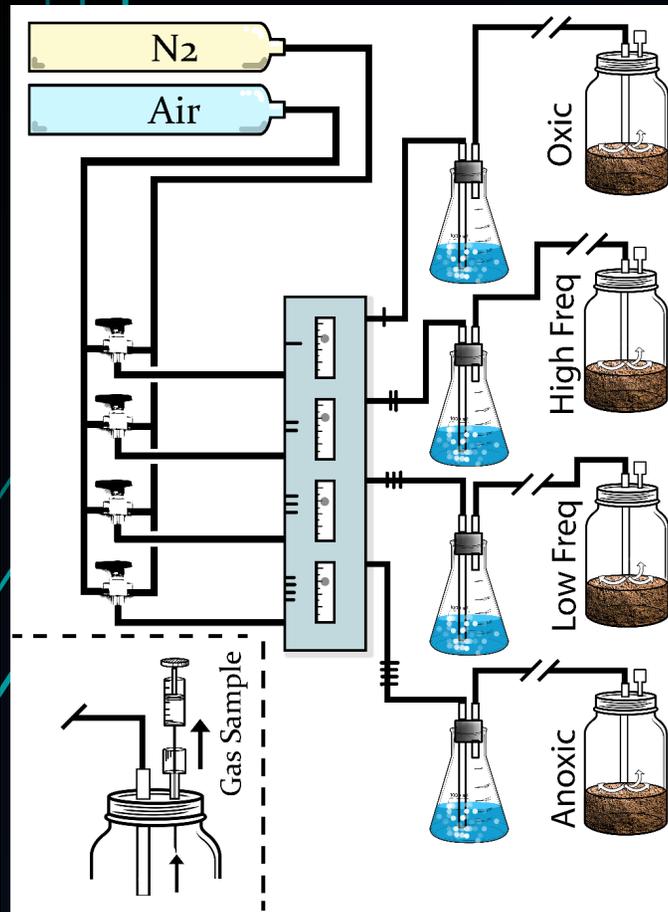


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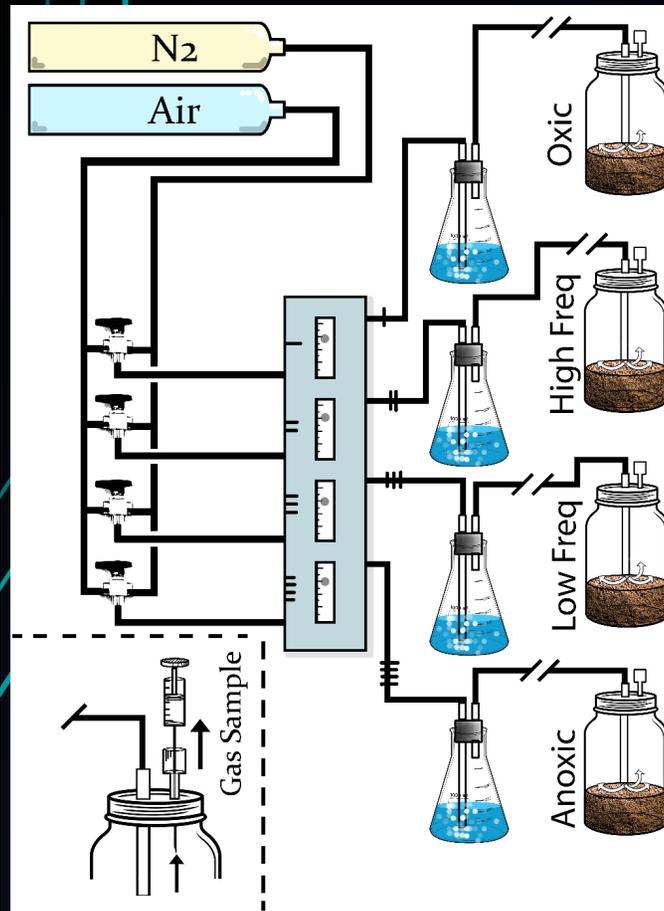


¹³C enriched plant biomass added to 20 g of soil



Figure credit: Alex Paya

Luquillo Experimental Forest, Puerto Rico



¹³C enriched plant biomass added to 20 g of soil

Treatments:

1. static oxic (**Oxic**)
2. 4-day-oxic/4-day-anoxic (**High frequency**)
3. 8-day-oxic/4-day-anoxic (**Low frequency**)
4. static anoxic (**Anoxic**)

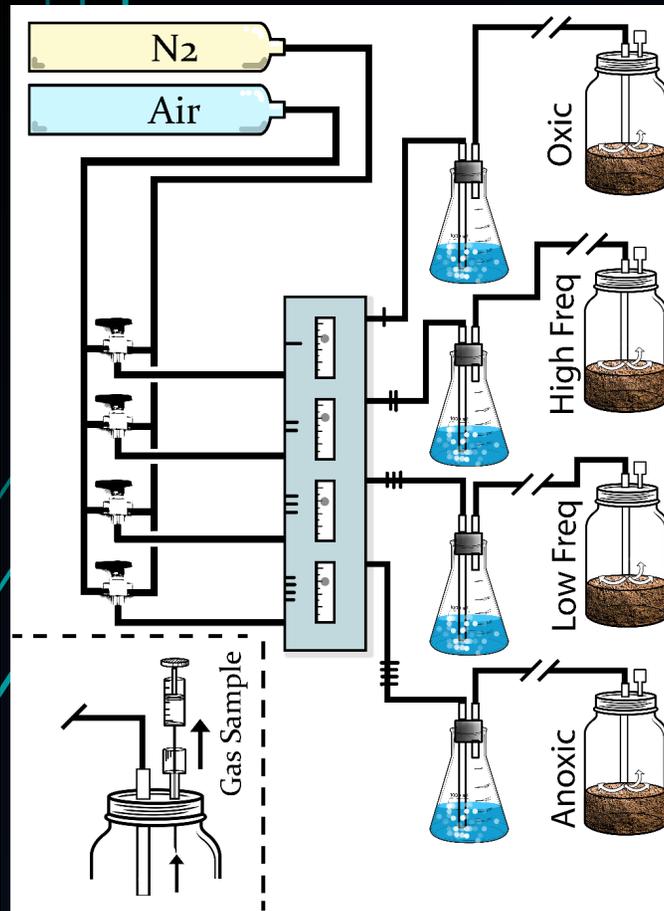
Oxic/anoxic conditions controlled by headspace

Oxic = air

Anoxic = N₂

Figure credit: Alex Paya

Luquillo Experimental Forest, Puerto Rico



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4. static anoxic (**Anoxic**)

Incubated for 44 days

85 SIP-fractionated metagenomes

10 bulk soil metagenomes

Figure credit: Alex Paya

Luquillo Experimental Forest, Puerto Rico

Overview

- 95 metagenomes

Luquillo Experimental Forest, Puerto Rico

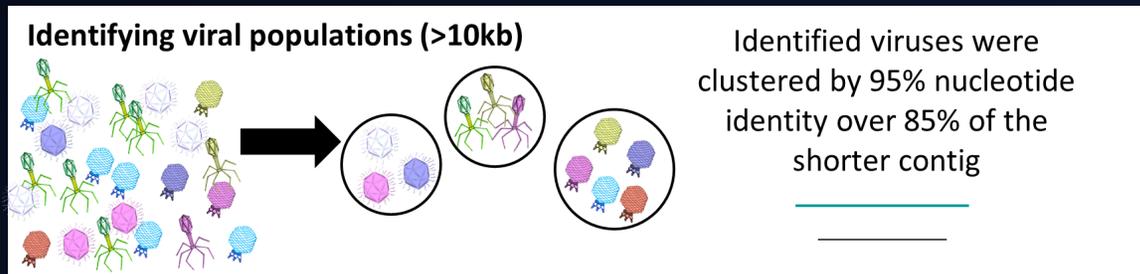
Overview

- 95 metagenomes
- 214 MAGs
 - 4 phyla of bacterial hosts:
 - *Acidobacteria*
 - *Actinobacteria*
 - *Bacteroidetes*
 - *Proteobacteria*

Luquillo Experimental Forest, Puerto Rico

Overview

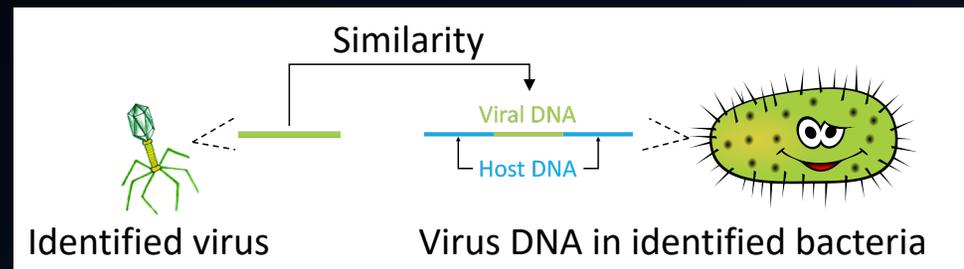
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- 640 vOTUs ($\geq 10\text{kb}$)



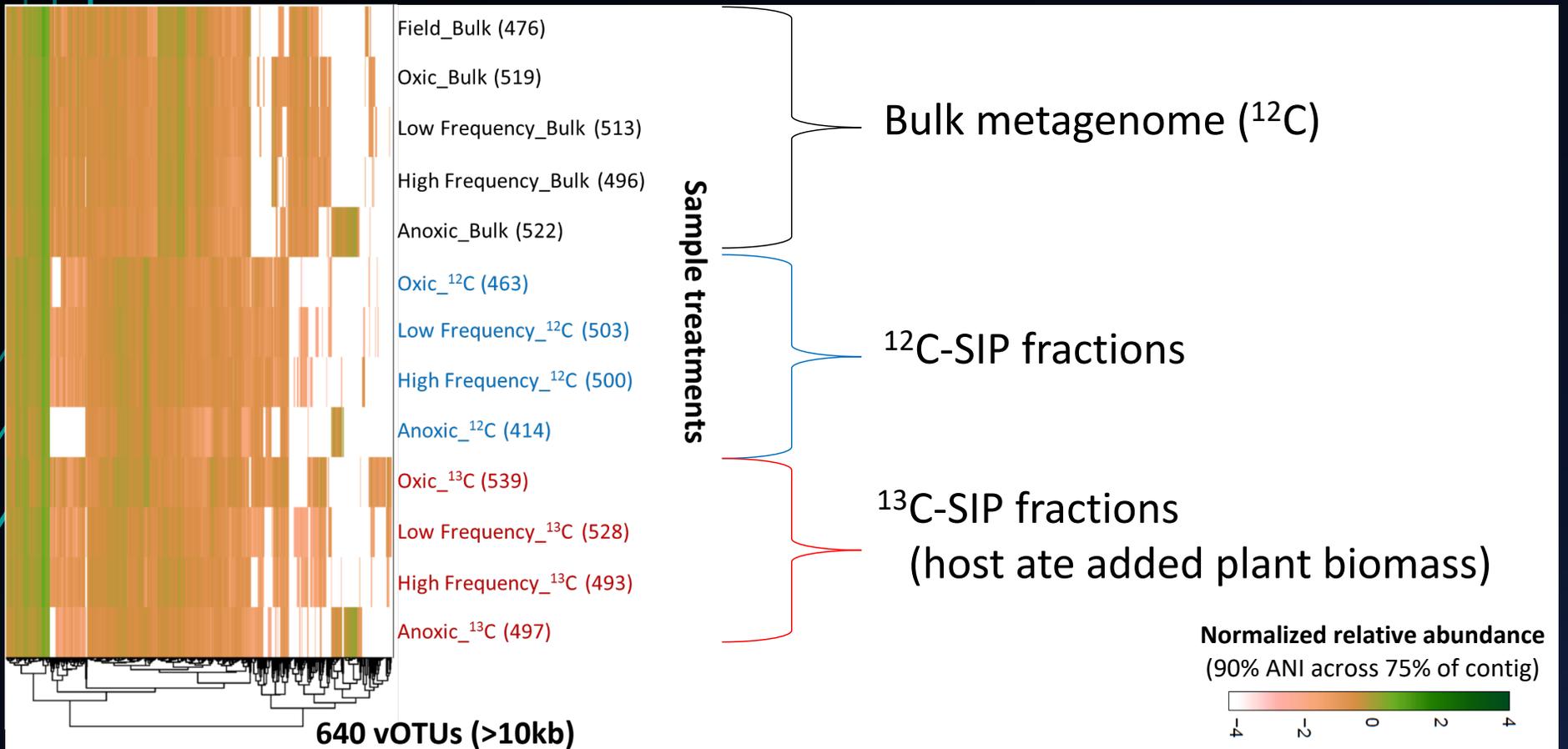
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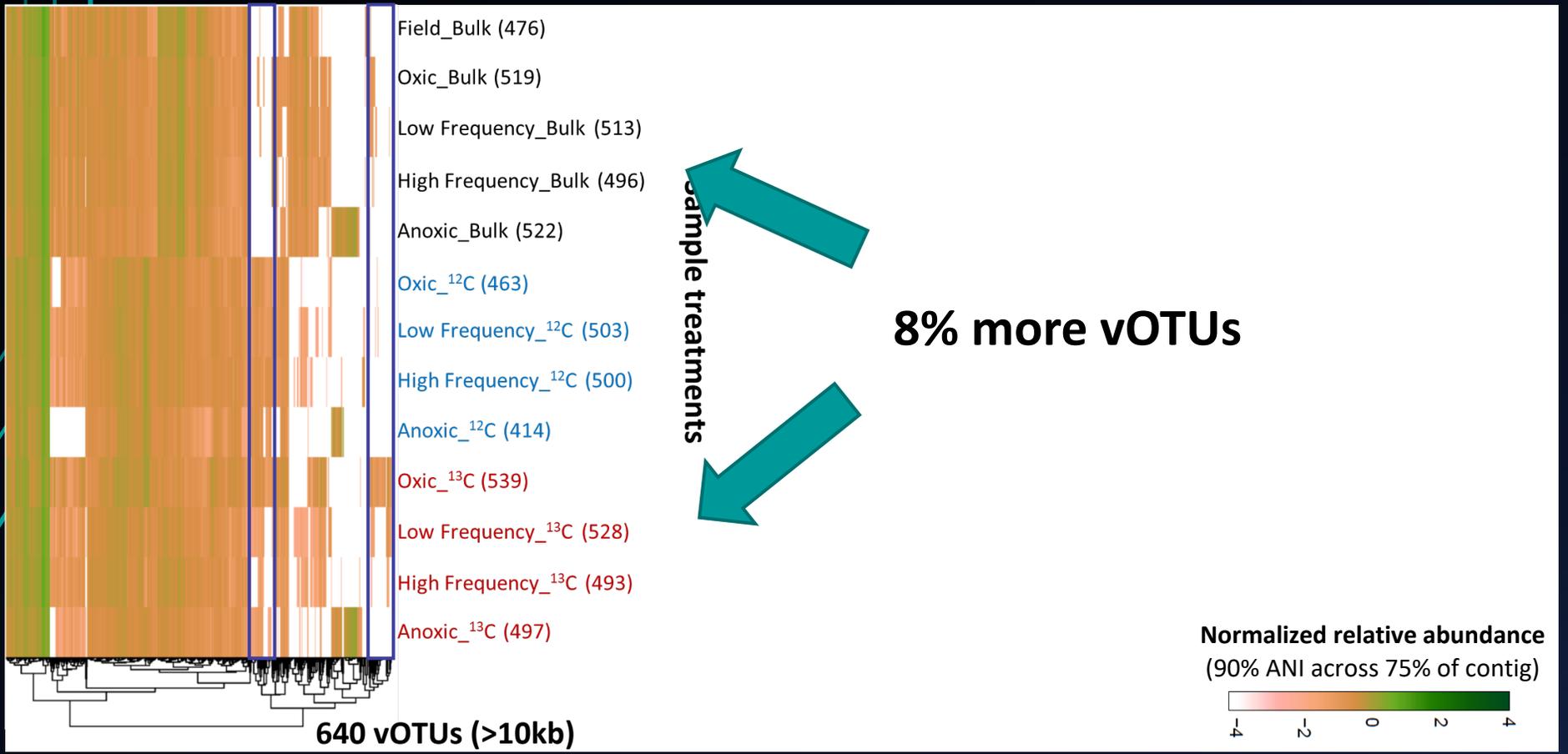
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& DeepVirFinder (score ≥ 0.9 and P value < 0.05)
- 640 vOTUs ($\geq 10\text{kb}$)
- 11% of viruses are linked to a host via nucleotide identity
(threshold 95% ANI & $\geq 1500\text{bp}$)



Luquillo Experimental Forest, Puerto Rico



SIP-fractions recovered more vOTUs

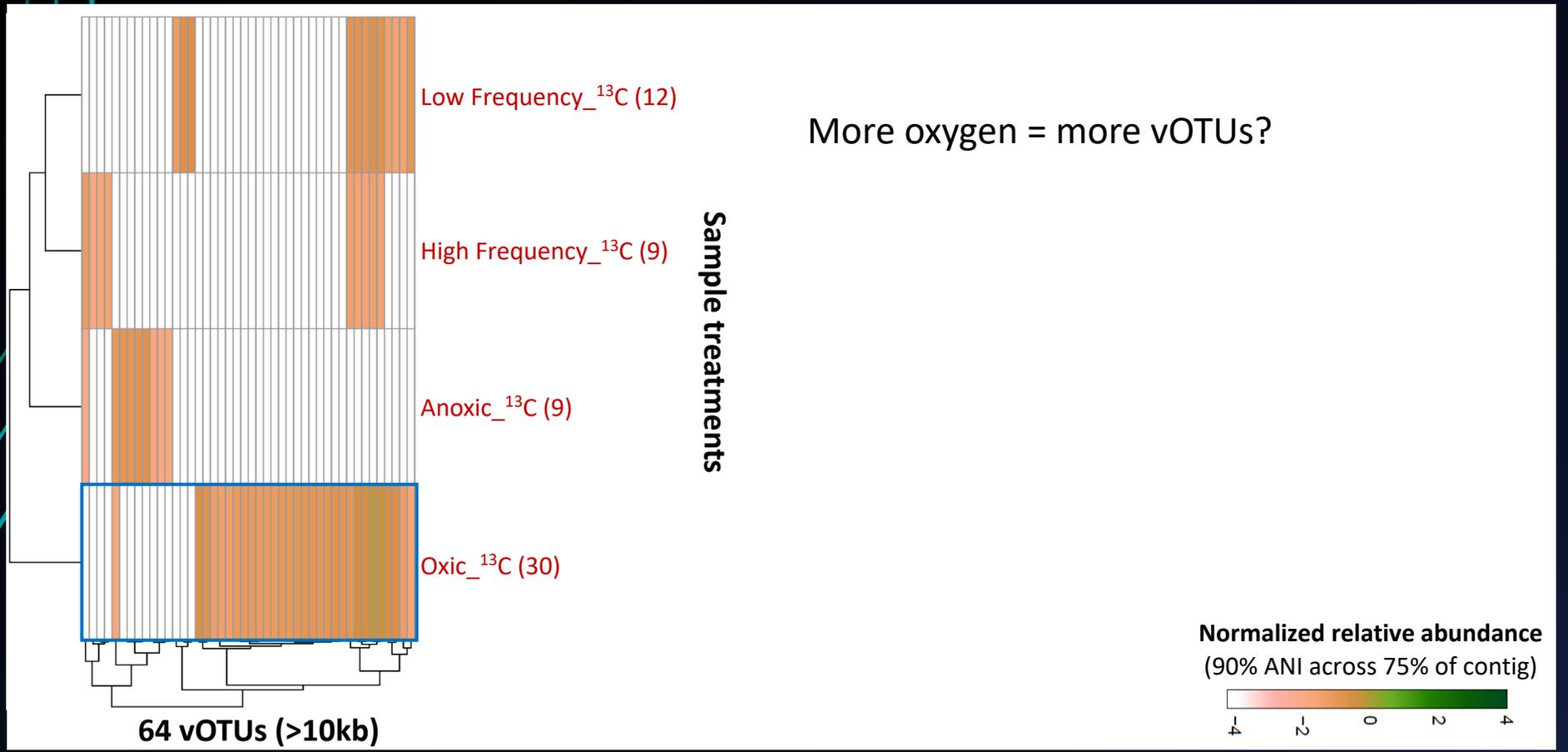


Focus on specific active viruses

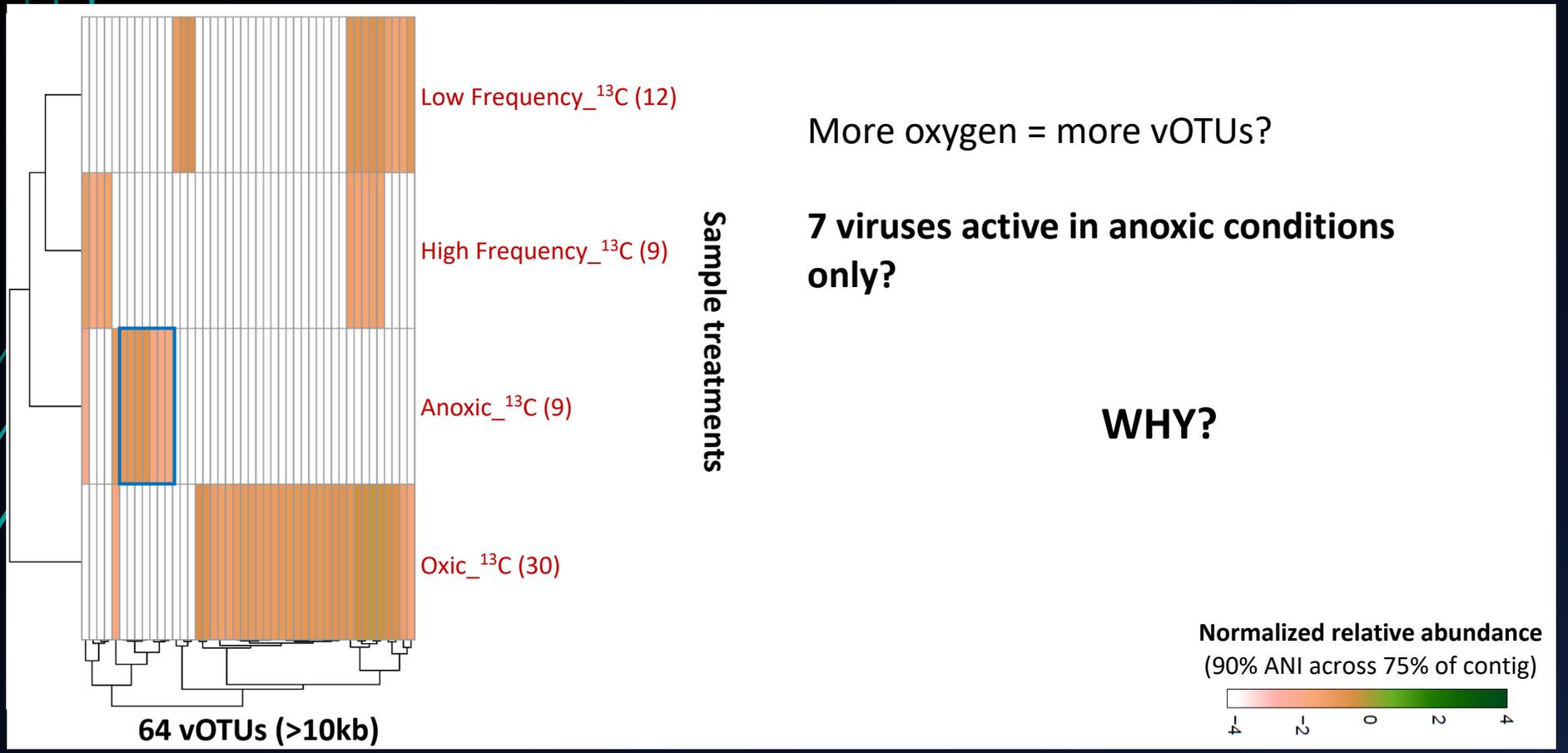


Redox conditions impact viral community

More oxygen = more vOTUs?



Redox conditions impact viral community



Summary

Applied SIP-metagenomics on two dramatically different LTER soils

1. Bonanza Creek, Alaska

- Identified microbes and viruses active in subzero temperatures
- Temporal succession of vOTUs
- Evidence that viruses may persist in the environment > 1 year

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- Linked viruses to key microbes involved in the degradation and fate of organic carbon compounds
- Redox strongly influences virus activity
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Synthesis

- A lot of novel viruses
- Tropical soils had 8x more sequencing, but only ~2x more vOTUs
 - Less organisms, reducing metagenome complexity?
- The metabolic repertoire was different between dormant/deceased & active microbes

Acknowledgments

Steve Blazewicz

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Neslihan Tas

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Janet Jansson

Mark Waldrop

LLNL group



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- US Geological Survey Mendenhall Fellowship
- DOE OBER Genomic Sciences Early Career Research Program award SCW1478

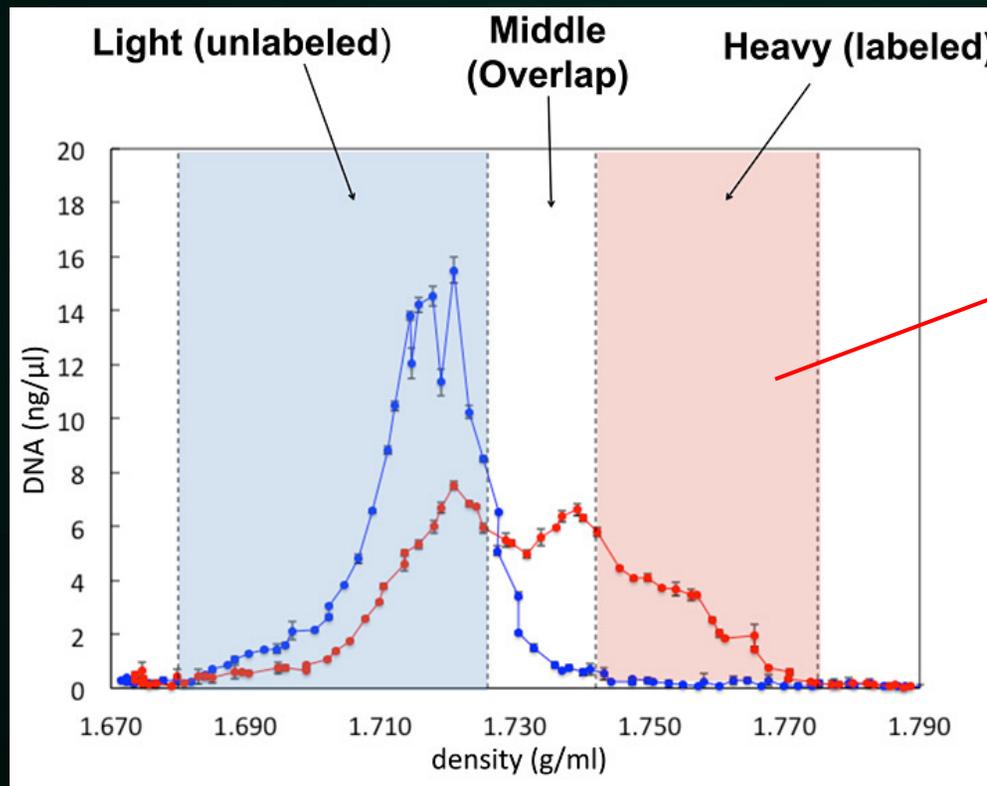


Thank You!

Ongoing work

🦠 Are viruses important...beyond being cool...for reducing uncertainty?

Using SIP to identify active viral populations



Active vOTUs?

How to do qSIP?